

**SECRETED AND TRANSMEMBRANE POLYPEPTIDES AND NUCLEIC ACIDS ENCODING  
THE SAME**

**FIELD OF THE INVENTION**

The present invention relates generally to the identification and isolation of novel DNA and to the  
5 recombinant production of novel polypeptides.

**BACKGROUND OF THE INVENTION**

Extracellular proteins play important roles in, among other things, the formation, differentiation and  
maintenance of multicellular organisms. The fate of many individual cells, e.g., proliferation, migration,  
10 differentiation, or interaction with other cells, is typically governed by information received from other cells  
and/or the immediate environment. This information is often transmitted by secreted polypeptides (for  
instance, mitogenic factors, survival factors, cytotoxic factors, differentiation factors, neuropeptides, and  
hormones) which are, in turn, received and interpreted by diverse cell receptors or membrane-bound proteins.  
These secreted polypeptides or signaling molecules normally pass through the cellular secretory pathway to  
15 reach their site of action in the extracellular environment.

Secreted proteins have various industrial applications, including as pharmaceuticals, diagnostics,  
biosensors and bioreactors. Most protein drugs available at present, such as thrombolytic agents, interferons,  
interleukins, erythropoietins, colony stimulating factors, and various other cytokines, are secretory proteins.  
Their receptors, which are membrane proteins, also have potential as therapeutic or diagnostic agents. Efforts  
20 are being undertaken by both industry and academia to identify new, native secreted proteins. Many efforts  
are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for  
novel secreted proteins. Examples of screening methods and techniques are described in the literature [see,  
for example, Klein et al., Proc. Natl. Acad. Sci. 93:7108-7113 (1996); U.S. Patent No. 5,536,637)].

Membrane-bound proteins and receptors can play important roles in, among other things, the  
25 formation, differentiation and maintenance of multicellular organisms. The fate of many individual cells, e.g.,  
proliferation, migration, differentiation, or interaction with other cells, is typically governed by information  
received from other cells and/or the immediate environment. This information is often transmitted by secreted  
polypeptides (for instance, mitogenic factors, survival factors, cytotoxic factors, differentiation factors,  
neuropeptides, and hormones) which are, in turn, received and interpreted by diverse cell receptors or  
30 membrane-bound proteins. Such membrane-bound proteins and cell receptors include, but are not limited to,  
cytokine receptors, receptor kinases, receptor phosphatases, receptors involved in cell-cell interactions, and  
cellular adhesion molecules like selectins and integrins. For instance, transduction of signals that regulate cell  
growth and differentiation is regulated in part by phosphorylation of various cellular proteins. Protein tyrosine  
kinases, enzymes that catalyze that process, can also act as growth factor receptors. Examples include

fibroblast growth factor receptor and nerve growth factor receptor.

Membrane-bound proteins and receptor molecules have various industrial applications, including as pharmaceutical and diagnostic agents. Receptor immunoadhesins, for instance, can be employed as therapeutic agents to block receptor-ligand interactions. The membrane-bound proteins can also be employed for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction.

5           Efforts are being undertaken by both industry and academia to identify new, native receptor or membrane-bound proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel receptor or membrane-bound proteins.

# 1.       **PRO196**

10           The abbreviations "TIE" or "tie" are acronyms, which stand for "tyrosine kinase containing Ig and EGF homology domains" and were coined to designate a new family of receptor tyrosine kinases which are almost exclusively expressed in vascular endothelial cells and early hemopoietic cells, and are characterized by the presence of an EGF-like domain, and extracellular folding units stabilized by intra-chain disulfide bonds, generally referred to as "immunoglobulin (IG)-like" folds. A tyrosine kinase homologous cDNA fragment  
15           from human leukemia cells (tie) was described by Partanen et al., Proc. Natl. Acad. Sci. USA **87**, 8913-8917 (1990). The mRNA of this human "tie" receptor has been detected in all human fetal and mouse embryonic tissues, and has been reported to be localized in the cardiac and vascular endothelial cells. Korhonen et al., Blood **80**, 2548-2555 (1992); PCT Application Publication No. WO 93/14124 (published 22 July 1993). The rat homolog of human tie, referred to as "tie-1", was identified by Maisonpierre et al., Oncogene **8**, 1631-1637  
20           (1993)). Another tie receptor, designated "tie-2" was originally identified in rats ( Dumont et al., Oncogene **8**, 1293-1301 (1993)), while the human homolog of tie-2, referred to as "ork" was described in U.S. Patent No. 5,447,860 (Ziegler). The murine homolog of tie-2 was originally termed "tek." The cloning of a mouse tie-2 receptor from a brain capillary cDNA library is disclosed in PCT Application Publication No. WO 95/13387 (published 18 May 1995). The TIE receptors are believed to be actively involved in angiogenesis,  
25           and may play a role in hemopoiesis as well.

          The expression cloning of human TIE-2 ligands has been described in PCT Application Publication No. WO 96/11269 (published 18 April 1996) and in U.S. Patent No. 5,521,073 (published 28 May 1996). A vector designated as  $\lambda$ gt10 encoding a TIE-2 ligand named "htie-2 ligand 1" or "hTL1" has been deposited under ATCC Accession No. 75928. A plasmid encoding another TIE-2 ligand designated "htie-2 2" or  
30           "hTL2" is available under ATCC Accession No. 75928. This second ligand has been described as an antagonist of the TAI-2 receptor. The identification of secreted human and mouse ligands for the TIE-2 receptor has been reported by Davis et al., Cell **87**, 1161-1169 (1996). The human ligand designated "Angiopoietin-1", to reflect its role in angiogenesis and potential action during hemopoiesis, is the same ligand as the ligand variously designated as "htie-2 1" or "hTL-1" in WO 96/11269. Angiopoietin-1 has been  
35           described to play an angiogenic role later and distinct from that of VEGF (Suri et al., Cell **87**, 1171-1180 (1996)). Since TIE-2 is apparently upregulated during the pathologic angiogenesis requisite for tumor growth (Kaipainen et al., Cancer Res. **54**, 6571-6577 (1994)) angiopoietin-1 has been suggested to be additionally

useful for specifically targeting tumor vasculature (Davis et al., supra).

2. **PRO444**

Efforts are being undertaken by both industry and academia to identify new, native secreted proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted proteins. We herein describe the identification and isolation of cDNA molecules encoding novel secreted polypeptides, designated herein as PRO444 polypeptides.

3. **PRO183**

Efforts are being undertaken by both industry and academia to identify new, native secreted proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted proteins. We herein describe the identification and isolation of cDNA molecules encoding novel polypeptides, designated herein as PRO183 polypeptides.

4. **PRO185**

Efforts are being undertaken by both industry and academia to identify new, native secreted proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted proteins. We herein describe the identification and isolation of cDNA molecules encoding novel polypeptides, designated herein as PRO185 polypeptides.

5. **PRO210 and PRO217**

Epidermal growth factor (EGF) is a conventional mitogenic factor that stimulates the proliferation of various types of cells including epithelial cells and fibroblasts. EGF binds to and activates the EGF receptor (EGFR), which initiates intracellular signaling and subsequent effects. The EGFR is expressed in neurons of the cerebral cortex, cerebellum, and hippocampus in addition to other regions of the central nervous system (CNS). In addition, EGF is also expressed in various regions of the CNS. Therefore, EGF acts not only on mitotic cells, but also on postmitotic neurons. In fact, many studies have indicated that EGF has neurotrophic or neuromodulatory effects on various types of neurons in the CNS. For example, EGF acts directly on cultured cerebral cortical and cerebellar neurons, enhancing neurite outgrowth and survival. On the other hand, EGF also acts on other cell types, including septal cholinergic and mesencephalic dopaminergic neurons, indirectly through glial cells. Evidence of the effects of EGF on neurons in the CNS is accumulating, but the mechanisms of action remain essentially unknown. EGF-induced signaling in mitotic cells is better understood than in postmitotic neurons. Studies of cloned pheochromocytoma PC12 cells and cultured cerebral cortical neurons have suggested that the EGF-induced neurotrophic actions are mediated by sustained activation of the EGFR and mitogen-activated protein kinase (MAPK) in response to EGF. The sustained intracellular signaling correlates with the decreased rate of EGFR down-regulation, which might determine the response of neuronal cells to EGF. It is likely that EGF is a multi-potent growth factor that acts upon various types of cells including mitotic cells and postmitotic neurons.

EGF is produced by the salivary and Brunner's glands of the gastrointestinal system, kidney, pancreas, thyroid gland, pituitary gland, and the nervous system, and is found in body fluids such as saliva, blood, cerebrospinal fluid (CSF), urine, amniotic fluid, prostatic fluid, pancreatic juice, and breast milk, Plata-Salaman, CR Peptides 12: 653-663 (1991).

EGF is mediated by its membrane specific receptor, which contains an intrinsic tyrosine kinase. Stoscheck CM et al., J. Cell Biochem. 31: 135-152 (1986). EGF is believed to function by binding to the extracellular portion of its receptor which induces a transmembrane signal that activates the intrinsic tyrosine kinase.

Purification and sequence analysis of the EGF-like domain has revealed the presence of six conserved cysteine residues which cross-bind to create three peptide loops, Savage CR et al., J. Biol. Chem. 248: 7669-7672 (1979). It is now generally known that several other peptides can react with the EGF receptor which share the same generalized motif  $X_nCX_7CX_4/5CX_{10}CX_5GX_2CX_n$ , where X represents any non-cysteine amino acid, and n is a variable repeat number. Non isolated peptides having this motif include TGF- $\alpha$ , amphiregulin, schwannoma-derived growth factor (SDGF), heparin-binding EGF-like growth factors and certain virally encoded peptides (e.g., Vaccinia virus, Reisner AH, Nature 313: 801-803 (1985), Shope fibroma virus, Chang W., et al., Mol Cell Biol. 7: 535-540 (1987), Molluscum contagiosum, Porter CD & Archard LC, J. Gen. Virol. 68: 673-682 (1987), and Myxoma virus, Upton C et al., J. Virol. 61: 1271-1275 (1987). Prigent SA & Lemoine N.R., Prog. Growth Factor Res. 4: 1-24 (1992).

EGF-like domains are not confined to growth factors but have been observed in a variety of cell-surface and extracellular proteins which have interesting properties in cell adhesion, protein-protein interaction and development, Laurence DJR & Gusterson BA, Tumor Biol. 11: 229-261 (1990). These proteins include blood coagulation factors (factors VI, IX, X, XII, protein C, protein S, protein Z, tissue plasminogen activator, urokinase), extracellular matrix components (laminin, cytactin, entactin), cell surface receptors (LDL receptor, thrombomodulin receptor) and immunity-related proteins (complement C1r, uromodulin).

Even more interesting, the general structure pattern of EGF-like precursors is preserved through lower organisms as well as in mammalian cells. A number of genes with developmental significance have been identified in invertebrates with EGF-like repeats. For example, the notch gene of Drosophila encodes 36 tandemly arranged 40 amino acid repeats which show homology to EGF, Wharton W et al., Cell 43: 557-581 (1985). Hydropathy plots indicate a putative membrane spanning domain, with the EGF-related sequences being located on the extracellular side of the membrane. Other homeotic genes with EGF-like repeats include Delta, 95F and 5ZD which were identified using probes based on Notch, and the nematode gene Lin-12 which encodes a putative receptor for a developmental signal transmitted between two specified cells.

Specifically, EGF has been shown to have potential in the preservation and maintenance of gastrointestinal mucosa and the repair of acute and chronic mucosal lesions, Konturek, PC et al., Eur. J. Gastroenterol Hepatol. 7 (10), 933-37 (1995), including the treatment of necrotizing enterocolitis, Zollinger-Ellison syndrome, gastrointestinal ulceration gastrointestinal ulcerations and congenital microvillus atrophy, A. Guglietta & PB Sullivan, Eur. J. Gastroenterol Hepatol, 7(10), 945-50 (1995). Additionally, EGF



has been implicated in hair follicle differentiation; C.L. du Cros, *J. Invest. Dermatol.* 101 (1 Suppl.), 106S-113S (1993), SG Hillier, *Clin. Endocrinol.* 33(4), 427-28 (1990); kidney function, L.L. Hamm et al., *Semin. Nephrol.* 13 (1): 109-15 (1993), RC Harris, *Am. J. Kidney Dis.* 17(6): 627-30 (1991); tear fluid, GB van Setten et al., *Int. Ophthalmol* 15(6); 359-62 (1991); vitamin K mediated blood coagulation, J. Stenflo et al., *Blood* 78(7): 1637-51 (1991). EGF is also implicated various skin disease characterized by abnormal keratinocyte differentiation, e.g., psoriasis, epithelial cancers such as squamous cell carcinomas of the lung, epidermoid carcinoma of the vulva and gliomas. King, LE et al., *Am. J. Med. Sci.* 296: 154-158 (1988).

Of great interest is mounting evidence that genetic alterations in growth factors signaling pathways are closely linked to developmental abnormalities and to chronic diseases including cancer. Aaronson SA, *Science* 254: 1146-1153 (1991). For example, c-erb-2 (also known as HER-2), a proto-oncogene with close structural similarity to EGF receptor protein, is overexpressed in human breast cancer. King et al., *Science* 229: 974-976 (1985); Gullick, WJ, *Hormones and their actions*, Cooke BA et al., eds, Amsterdam, Elsevier, pp 349-360 (1986).

## 6. PRO215

Protein-protein interactions include receptor and antigen complexes and signaling mechanisms. As more is known about the structural and functional mechanisms underlying protein-protein interactions, protein-protein interactions can be more easily manipulated to regulate the particular result of the protein-protein interaction. Thus, the underlying mechanisms of protein-protein interactions are of interest to the scientific and medical community.

All proteins containing leucine-rich repeats are thought to be involved in protein-protein interactions. Leucine-rich repeats are short sequence motifs present in a number of proteins with diverse functions and cellular locations. The crystal structure of ribonuclease inhibitor protein has revealed that leucine-rich repeats correspond to beta-alpha structural units. These units are arranged so that they form a parallel beta-sheet with one surface exposed to solvent, so that the protein acquires an unusual, nonglobular shape. These two features have been indicated as responsible for the protein-binding functions of proteins containing leucine-rich repeats. See, Kobe and Deisenhofer, *Trends Biochem. Sci.*, 19(10):415-421 (Oct. 1994).

A study has been reported on leucine-rich proteoglycans which serve as tissue organizers, orienting and ordering collagen fibrils during ontogeny and are involved in pathological processes such as wound healing, tissue repair, and tumor stroma formation. Iozzo, R. V., *Crit. Rev. Biochem. Mol. Biol.*, 32(2):141-174 (1997). Others studies implicating leucine rich proteins in wound healing and tissue repair are De La Salle, C., et al., *Vouv. Rev. Fr. Hematol.* (Germany), 37(4):215-222 (1995), reporting mutations in the leucine rich motif in a complex associated with the bleeding disorder Bernard-Soulier syndrome and Chlmetson, K. J., *Thromb. Haemost.* (Germany), 74(1):111-116 (July 1995), reporting that platelets have leucine rich repeats. Another protein of particular interest which has been reported to have leucine-rich repeats is the SLIT protein which has been reported to be useful in treating neuro-degenerative diseases such as Alzheimer's disease, nerve damage such as in Parkinson's disease, and for diagnosis of cancer, see, Artavanistsakonas, S. and Rothberg, J. M., WO9210518-A1 by Yale University. Other studies reporting on

the biological functions of proteins having leucine-rich repeats include: Tayar, N., et al., Mol. Cell Endocrinol., (Ireland), 125(1-2):65-70 (Dec. 1996) (gonadotropin receptor involvement); Miura, Y., et al., Nippon Rinsho (Japan), 54(7):1784-1789 (July 1996) (apoptosis involvement); Harris, P. C., et al., J. Am. Soc. Nephrol., 6(4):1125-1133 (Oct. 1995) (kidney disease involvement); and Ruoslahti, E. I., et al., WO9110727-A by La Jolla Cancer Research Foundation (decorin binding to transforming growth factor $\beta$  involvement for treatment for cancer, wound healing and scarring).

#### 7. PRO242, PRO1318 and PRO1600

Leukocytes include monocytes, macrophages, basophils, and eosinophils and play an important role in the immune response. These cells are important in the mechanisms initiated by T and/or B lymphocytes and secrete a range of cytokines which recruit and activate other inflammatory cells and contribute to tissue destruction.

Thus, investigation of the regulatory processes by which leukocytes move to their appropriate destination and interact with other cells is critical. Currently, leukocytes are thought to move from the blood to injured or inflamed tissues by rolling along the endothelial cells of the blood vessel wall. This movement is mediated by transient interactions between selectins and their ligands. Next, the leukocyte must move through the vessel wall and into the tissues. This diapedesis and extravasation step involves cell activation which promotes a more stable leukocyte-endothelial cell interaction, again mediated by integrins and their ligands.

Chemokines are a large family of structurally related polypeptide cytokines. These molecules stimulate leukocyte movement and may explain leukocyte trafficking in different inflammatory situations. Chemokines mediate the expression of particular adhesion molecules on endothelial cells, and they produce chemoattractants which activate specific cell types. In addition, the chemokines stimulate proliferation and regulate activation of specific cell types. In both of these activities, chemokines demonstrate a high degree of target cell specificity.

The chemokine family is divided into two subfamilies based on whether two amino terminal cysteine residues are immediately adjacent (C-C) or separated by one amino acid (C-X-C). Chemokines of the C-X-C family generally activate neutrophils and fibroblasts while the C-C chemokines act on a more diverse group of target cells including monocytes/macrophages, basophils, eosinophils and T lymphocytes. The known chemokines of both subfamilies are synthesized by many diverse cell types as reviewed in Thomson A. (1994) *The Cytokine Handbook*, 2 d Ed. Academic Press, N.Y.

Known chemokines include macrophage inflammatory proteins alpha and beta (MIP-1 alpha and beta), I-309, RANTES, and monocyte chemotactic protein (MCP-1).

MIP-1 alpha and MIP-1 beta were first purified from a stimulated mouse macrophage cell line and elicited an inflammatory response when injected into normal tissues. MIP-1 alpha and MIP-1 beta consist of 68-69 amino acids and share approximately 70% identity in their mature secreted forms. Both are expressed in T cells, B cells and monocytes which are stimulated by mitogens, anti-CD3 and endotoxin, and both polypeptides bind heparin and stimulate monocytes. MIP-1 alpha acts as a chemoattractant for the CD-8 subset

of T lymphocytes and eosinophils, while MIP-1 beta chemoattracts the CD-4 subset of T lymphocytes. In addition, these proteins are known to stimulate myelopoiesis in mice.

RANTES is regulated by interleukins-1 and -4, transforming nerve factor and interferon- gamma and is expressed in T cells, platelets, stimulated rheumatoid synovial fibroblasts, and in some tumor cell lines. RANTES affects lymphocytes, monocytes, basophils and eosinophils. RANTES expression is substantially reduced upon T cell stimulation.

Monocyte chemotactic protein (MCP-1) is a 76 amino acid protein which appears to be expressed in almost all cells and tissues upon stimulation by a variety of agents. However, the targets of MCP-1 are limited to monocytes and basophils. In these cells, MCP-1 induces a MCP-1 receptor. Two related proteins, MCP-2 and MCP-3, have 62% and 73% identity, respectively, with MCP-1 and share its chemoattractant specificity or monocytes.

Current techniques for diagnosis of abnormalities in inflamed or diseased tissues mainly rely on observation of clinical symptoms or serological analyses of body tissues or fluids for hormones, polypeptides or various metabolites. Problems exist with these diagnostic techniques. First, patients may not manifest clinical symptoms at early stages of disease. Second, serological tests do not always differentiate between invasive diseases and genetic syndromes. Thus, the identification of expressed chemokines is important to the development of new diagnostic techniques, effective therapies, and to aid in the understanding of molecular pathogenesis.

The chemokine molecules were reviewed in Schall TJ (1994) Chemotactic Cytokines: Targets for Therapeutic Development. International Business Communications, Southborough Mass. pp 180-270; and in Paul WE (1993) Fundamental Immunology, 3rd Ed. Raven Press, N.Y. pp 822-826.

## 8. PRO288

Control of cell numbers in mammals is believed to be determined, in part, by a balance between cell proliferation and cell death. One form of cell death, sometimes referred to as necrotic cell death, is typically characterized as a pathologic form of cell death resulting from some trauma or cellular injury. In contrast, there is another, "physiologic" form of cell death which usually proceeds in an orderly or controlled manner. This orderly or controlled form of cell death is often referred to as "apoptosis" [see, e.g., Barr et al., Bio/Technology, 12:487-493 (1994); Steller et al., Science, 267:1445-1449 (1995)]. Apoptotic cell death naturally occurs in many physiological processes, including embryonic development and clonal selection in the immune system [Itoh et al., Cell, 66:233-243 (1991)]. Decreased levels of apoptotic cell death have been associated with a variety of pathological conditions, including cancer, lupus, and herpes virus infection [Thompson, Science, 267:1456-1462 (1995)]. Increased levels of apoptotic cell death may be associated with a variety of other pathological conditions, including AIDS, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, retinitis pigmentosa, cerebellar degeneration, aplastic anemia, myocardial infarction, stroke, reperfusion injury, and toxin-induced liver disease [see, Thompson, supra].

Apoptotic cell death is typically accompanied by one or more characteristic morphological and biochemical changes in cells, such as condensation of cytoplasm, loss of plasma membrane microvilli,

segmentation of the nucleus, degradation of chromosomal DNA or loss of mitochondrial function. A variety of extrinsic and intrinsic signals are believed to trigger or induce such morphological and biochemical cellular changes [Raff, Nature, **356**:397-400 (1992); Steller, supra; Sachs et al., Blood, **82**:15 (1993)]. For instance, they can be triggered by hormonal stimuli, such as glucocorticoid hormones for immature thymocytes, as well as withdrawal of certain growth factors [Watanabe-Fukunaga et al., Nature, **356**:314-317 (1992)]. Also, some identified oncogenes such as *myc*, *rel*, and *E1A*, and tumor suppressors, like *p53*, have been reported to have a role in inducing apoptosis. Certain chemotherapy drugs and some forms of radiation have likewise been observed to have apoptosis-inducing activity [Thompson, supra].

Various molecules, such as tumor necrosis factor- $\alpha$  ("TNF- $\alpha$ "), tumor necrosis factor- $\beta$  ("TNF- $\beta$ " or "lymphotoxin"), CD30 ligand, CD27 ligand, CD40 ligand, OX-40 ligand, 4-1BB ligand, Apo-1 ligand (also referred to as Fas ligand or CD95 ligand), and Apo-2 ligand (also referred to as TRAIL) have been identified as members of the tumor necrosis factor ("TNF") family of cytokines [See, e.g., Gruss and Dower, Blood, **85**:3378-3404 (1995); Wiley et al., Immunity, **3**:673-682 (1995); Pitti et al., J. Biol. Chem., **271**:12687-12690 (1996)]. Among these molecules, TNF- $\alpha$ , TNF- $\beta$ , CD30 ligand, 4-1BB ligand, Apo-1 ligand, and Apo-2 ligand (TRAIL) have been reported to be involved in apoptotic cell death. Both TNF- $\alpha$  and TNF- $\beta$  have been reported to induce apoptotic death in susceptible tumor cells [Schmid et al., Proc. Natl. Acad. Sci., **83**:1881 (1986); Dealtry et al., Eur. J. Immunol., **17**:689 (1987)]. Zheng et al. have reported that TNF- $\alpha$  is involved in post-stimulation apoptosis of CD8-positive T cells [Zheng et al., Nature, **377**:348-351 (1995)]. Other investigators have reported that CD30 ligand may be involved in deletion of self-reactive T cells in the thymus [Amakawa et al., Cold Spring Harbor Laboratory Symposium on Programmed Cell Death, Abstr. No. 10, (1995)].

Mutations in the mouse Fas/Apo-1 receptor or ligand genes (called *lpr* and *gld*, respectively) have been associated with some autoimmune disorders, indicating that Apo-1 ligand may play a role in regulating the clonal deletion of self-reactive lymphocytes in the periphery [Krammer et al., Curr. Op. Immunol., **6**:279-289 (1994); Nagata et al., Science, **267**:1449-1456 (1995)]. Apo-1 ligand is also reported to induce post-stimulation apoptosis in CD4-positive T lymphocytes and in B lymphocytes, and may be involved in the elimination of activated lymphocytes when their function is no longer needed [Krammer et al., supra; Nagata et al., supra]. Agonist mouse monoclonal antibodies specifically binding to the Apo-1 receptor have been reported to exhibit cell killing activity that is comparable to or similar to that of TNF- $\alpha$  [Yonehara et al., J. Exp. Med., **169**:1747-1756 (1989)].

Induction of various cellular responses mediated by such TNF family cytokines is believed to be initiated by their binding to specific cell receptors. Two distinct TNF receptors of approximately 55-kDa (TNFR1) and 75-kDa (TNFR2) have been identified [Hohman et al., J. Biol. Chem., **264**:14927-14934 (1989); Brockhaus et al., Proc. Natl. Acad. Sci., **87**:3127-3131 (1990); EP 417,563, published March 20, 1991] and human and mouse cDNAs corresponding to both receptor types have been isolated and characterized [Loetscher et al., Cell, **61**:351 (1990); Schall et al., Cell, **61**:361 (1990); Smith et al., Science, **248**:1019-1023 (1990); Lewis et al., Proc. Natl. Acad. Sci., **88**:2830-2834 (1991); Goodwin et al., Mol. Cell. Biol., **11**:3020-3026 (1991)]. Extensive polymorphisms have been associated with both TNF receptor genes [see, e.g., Takao et

al., *Immunogenetics*, **37**:199-203 (1993)]. Both TNFRs share the typical structure of cell surface receptors including extracellular, transmembrane and intracellular regions. The extracellular portions of both receptors are found naturally also as soluble TNF-binding proteins [Nophar, Y. et al., *EMBO J.*, **9**:3269 (1990); and Kohno, T. et al., *Proc. Natl. Acad. Sci. U.S.A.*, **87**:8331 (1990)]. More recently, the cloning of recombinant soluble TNF receptors was reported by Hale et al. [*J. Cell. Biochem. Supplement* **15F**, 1991, p. 113 (P424)].

5 The extracellular portion of type 1 and type 2 TNFRs (TNFR1 and TNFR2) contains a repetitive amino acid sequence pattern of four cysteine-rich domains (CRDs) designated 1 through 4, starting from the NH<sub>2</sub>-terminus. Each CRD is about 40 amino acids long and contains 4 to 6 cysteine residues at positions which are well conserved [Schall et al., *supra*; Loetscher et al., *supra*; Smith et al., *supra*; Nophar et al., *supra*; Kohno et al., *supra*]. In TNFR1, the approximate boundaries of the four CRDs are as follows: CRD1-  
10 amino acids 14 to about 53; CRD2- amino acids from about 54 to about 97; CRD3- amino acids from about 98 to about 138; CRD4- amino acids from about 139 to about 167. In TNFR2, CRD1 includes amino acids 17 to about 54; CRD2- amino acids from about 55 to about 97; CRD3- amino acids from about 98 to about 140; and CRD4- amino acids from about 141 to about 179 [Banner et al., *Cell*, **73**:431-435 (1993)]. The potential role of the CRDs in ligand binding is also described by Banner et al., *supra*.

15 A similar repetitive pattern of CRDs exists in several other cell-surface proteins, including the p75 nerve growth factor receptor (NGFR) [Johnson et al., *Cell*, **47**:545 (1986); Radeke et al., *Nature*, **325**:593 (1987)], the B cell antigen CD40 [Stamenkovic et al., *EMBO J.*, **8**:1403 (1989)], the T cell antigen OX40 [Mallet et al., *EMBO J.*, **9**:1063 (1990)] and the Fas antigen [Yonchara et al., *supra* and Itoh et al., *supra*]. CRDs are also found in the soluble TNFR (sTNFR)-like T2 proteins of the Shope and myxoma poxviruses  
20 [Upton et al., *Virology*, **160**:20-29 (1987); Smith et al., *Biochem. Biophys. Res. Commun.*, **176**:335 (1991); Upton et al., *Virology*, **184**:370 (1991)]. Optimal alignment of these sequences indicates that the positions of the cysteine residues are well conserved. These receptors are sometimes collectively referred to as members of the TNF/NGF receptor superfamily. Recent studies on p75NGFR showed that the deletion of CRD1 [Welcher, A.A. et al., *Proc. Natl. Acad. Sci. USA*, **88**:159-163 (1991)] or a 5-amino acid insertion in this  
25 domain [Yan, H. and Chao, M.V., *J. Biol. Chem.*, **266**:12099-12104 (1991)] had little or no effect on NGF binding [Yan, H. and Chao, M.V., *supra*]. p75 NGFR contains a proline-rich stretch of about 60 amino acids, between its CRD4 and transmembrane region, which is not involved in NGF binding [Peetre, C. et al., *Eur. J. Hematol.*, **41**:414-419 (1988); Seckinger, P. et al., *J. Biol. Chem.*, **264**:11966-11973 (1989); Yan, H. and Chao, M.V., *supra*]. A similar proline-rich region is found in TNFR2 but not in TNFR1.

30 Itoh et al. disclose that the Apo-1 receptor can signal an apoptotic cell death similar to that signaled by the 55-kDa TNFR1 [Itoh et al., *supra*]. Expression of the Apo-1 antigen has also been reported to be down-regulated along with that of TNFR1 when cells are treated with either TNF- $\alpha$  or anti-Apo-1 mouse monoclonal antibody [Krammer et al., *supra*; Nagata et al., *supra*]. Accordingly, some investigators have hypothesized that cell lines that co-express both Apo-1 and TNFR1 receptors may mediate cell killing through  
35 common signaling pathways [*Id.*].

The TNF family ligands identified to date, with the exception of lymphotoxin- $\alpha$ , are type II transmembrane proteins, whose C-terminus is extracellular. In contrast, the receptors in the TNF receptor

(TNFR) family identified to date are type I transmembrane proteins. In both the TNF ligand and receptor families, however, homology identified between family members has been found mainly in the extracellular domain ("ECD"). Several of the TNF family cytokines, including TNF- $\alpha$ , Apo-1 ligand and CD40 ligand, are cleaved proteolytically at the cell surface; the resulting protein in each case typically forms a homotrimeric molecule that functions as a soluble cytokine. TNF receptor family proteins are also usually cleaved proteolytically to release soluble receptor ECDs that can function as inhibitors of the cognate cytokines.

Recently, other members of the TNFR family have been identified. In Marsters et al., Curr. Biol., 6:750 (1996), investigators describe a full length native sequence human polypeptide, called Apo-3, which exhibits similarity to the TNFR family in its extracellular cysteine-rich repeats and resembles TNFR1 and CD95 in that it contains a cytoplasmic death domain sequence [see also Marsters et al., Curr. Biol., 6:1669 (1996)]. Apo-3 has also been referred to by other investigators as DR3, wsl-1 and TRAMP [Chinnaiyan et al., Science, 274:990 (1996); Kitson et al., Nature, 384:372 (1996); Bodmer et al., Immunity, 6:79 (1997)].

Pan et al. have disclosed another TNF receptor family member referred to as "DR4" [Pan et al., Science, 276:111-113 (1997)]. The DR4 was reported to contain a cytoplasmic death domain capable of engaging the cell suicide apparatus. Pan et al. disclose that DR4 is believed to be a receptor for the ligand known as Apo-2 ligand or TRAIL.

In Sheridan et al., Science, 277:818-821 (1997) and Pan et al., Science, 277:815-818 (1997), another molecule believed to be a receptor for the Apo-2 ligand (TRAIL) is described. That molecule is referred to as DR5 (it has also been alternatively referred to as Apo-2). Like DR4, DR5 is reported to contain a cytoplasmic death domain and be capable of signaling apoptosis.

In Sheridan et al., supra, a receptor called DcR1 (or alternatively, Apo-2DcR) is disclosed as being a potential decoy receptor for Apo-2 ligand (TRAIL). Sheridan et al. report that DcR1 can inhibit Apo-2 ligand function *in vitro*. See also, Pan et al., supra, for disclosure on the decoy receptor referred to as TRID.

As presently understood, the cell death program contains at least three important elements - activators, inhibitors, and effectors; in *C. elegans*, these elements are encoded respectively by three genes, *Ced-4*, *Ced-9* and *Ced-3* [Steller, Science, 267:1445 (1995); Chinnaiyan et al., Science, 275:1122-1126 (1997); Wang et al., Cell, 90:1-20 (1997)]. Two of the TNFR family members, TNFR1 and Fas/Apo1 (CD95), can activate apoptotic cell death [Chinnaiyan and Dixit, Current Biology, 6:555-562 (1996); Fraser and Evan, Cell, 85:781-784 (1996)]. TNFR1 is also known to mediate activation of the transcription factor, NF- $\kappa$ B [Tartaglia et al., Cell, 74:845-853 (1993); Hsu et al., Cell, 84:299-308 (1996)]. In addition to some ECD homology, these two receptors share homology in their intracellular domain (ICD) in an oligomerization interface known as the death domain [Tartaglia et al., supra; Nagata, Cell, 88:355 (1997)]. Death domains are also found in several metazoan proteins that regulate apoptosis, namely, the Drosophila protein, Reaper, and the mammalian proteins referred to as FADD/MORT1, TRADD, and RIP [Cleaveland and Ihle, Cell, 81:479-482 (1995)]. Using the yeast-two hybrid system, Raven et al. report the identification of protein, wsl-1, which binds to the TNFR1 death domain [Raven et al., Programmed Cell Death Meeting, September 20-24, 1995, Abstract at page 127; Raven et al., European Cytokine Network, 7:Abstr. 82 at page 210 (April-June 1996); see also, Kitson et al., Nature, 384:372-375 (1996)]. The wsl-1 protein is described as being homologous to TNFR1 (48% identity)

and having a restricted tissue distribution. According to Raven et al., the tissue distribution of wsl-1 is significantly different from the TNFR1 binding protein, TRADD.

Upon ligand binding and receptor clustering, TNFR1 and CD95 are believed to recruit FADD into a death-inducing signalling complex. CD95 purportedly binds FADD directly, while TNFR1 binds FADD indirectly via TRADD [Chinnaiyan et al., *Cell*, **81**:505-512 (1995); Boldin et al., *J. Biol. Chem.*, **270**:387-391 (1995); Hsu et al., *supra*; Chinnaiyan et al., *J. Biol. Chem.*, **271**:4961-4965 (1996)]. It has been reported that FADD serves as an adaptor protein which recruits the *Ced-3*-related protease, MACH $\alpha$ /FLICE (caspase 8), into the death signalling complex [Boldin et al., *Cell*, **85**:803-815 (1996); Muzio et al., *Cell*, **85**:817-827 (1996)]. MACH $\alpha$ /FLICE appears to be the trigger that sets off a cascade of apoptotic proteases, including the interleukin-1 $\beta$  converting enzyme (ICE) and CPP32/Yama, which may execute some critical aspects of the cell death programme [Fraser and Evan, *supra*].

It was recently disclosed that programmed cell death involves the activity of members of a family of cysteine proteases related to the *C. elegans* cell death gene, *ced-3*, and to the mammalian IL-1-converting enzyme, ICE. The activity of the ICE and CPP32/Yama proteases can be inhibited by the product of the cowpox virus gene, *crmA* [Ray et al., *Cell*, **69**:597-604 (1992); Tewari et al., *Cell*, **81**:801-809 (1995)]. Recent studies show that CrmA can inhibit TNFR1- and CD95-induced cell death [Enari et al., *Nature*, **375**:78-81 (1995); Tewari et al., *J. Biol. Chem.*, **270**:3255-3260 (1995)].

As reviewed recently by Tewari et al., TNFR1, TNFR2 and CD40 modulate the expression of proinflammatory and costimulatory cytokines, cytokine receptors, and cell adhesion molecules through activation of the transcription factor, NF- $\kappa$ B [Tewari et al., *Curr. Op. Genet. Develop.*, **6**:39-44 (1996)]. NF- $\kappa$ B is the prototype of a family of dimeric transcription factors whose subunits contain conserved Rel regions [Verma et al., *Genes Develop.*, **9**:2723-2735 (1996); Baldwin, *Ann. Rev. Immunol.*, **14**:649-681 (1996)]. In its latent form, NF- $\kappa$ B is complexed with members of the I $\kappa$ B inhibitor family; upon inactivation of the I $\kappa$ B in response to certain stimuli, released NF- $\kappa$ B translocates to the nucleus where it binds to specific DNA sequences and activates gene transcription.

For a review of the TNF family of cytokines and their receptors, see Gruss and Dower, *supra*.

## 9. PRO365

Polypeptides such as human 2-19 protein may function as cytokines. Cytokines are low molecular weight proteins which function to stimulate or inhibit the differentiation, proliferation or function of immune cells. Cytokines often act as intercellular messengers and have multiple physiological effects. Given the physiological importance of immune mechanisms *in vivo*, efforts are currently being under taken to identify new, native proteins which are involved in effecting the immune system. We describe herein the identification of a novel polypeptide which has homology to the human 2-19 protein.

## 10. PRO1361

Efforts are being undertaken by both industry and academia to identify new, native transmembrane receptor proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to

identify the coding sequences for novel receptor proteins. We herein describe the identification and characterization of novel transmembrane polypeptides, designated herein as PRO1361 polypeptides.

#### 11. PRO1308

Follistatin is a secreted protein that regulates secretion of pituitary follicle-stimulating hormone (FSH). It functions by binding to, and thereby inhibiting, proteins such as activin and other members of the transforming growth factor beta (TGF $\beta$ ) family, that stimulate the production and secretion of FSH from the anterior pituitary. Follistatin is also involved in mechanisms that control basic development, including the induction of neural development. Follistatin also exhibits angiogenic properties, particularly in combination with basic fibroblast growth factor (bFGF). As such, there is strong interest in identifying new members of the follistatin family of proteins. The identification and characterization of follistatins is the topic of the following references which are incorporated herein by reference: Sugino *et al.*, J. Med Invest (1997) 44:(1-2):1-14; Mather *et al.*, Proc. Soc. Exp. Biol. Med. (1997) 215(3):209-222; Thomsen, G.H., Trends Genet (1997) 13(6): 209-211; DePaolo, L.V., Proc. Soc. Exp. Biol. Med. (1997) 214(4):328-339; Peng *et al.*, Biol. Signals (1996) 5(2):81-89, and Halvorson *et al.*, Fertil Steril (1996) 65(3):459-469.

#### 12. PRO1183

Protoporphyrinogen oxidase catalyzes the penultimate step in the heme biosynthetic pathway. Deficiency in activity of this enzyme results in the human genetic disease variegate porphyria. Thus, protoporphyrinogen oxidases and molecules which either modulate or are related to these oxidases are of interest. Moreover, oxidases, and related molecules in general are also of interest. Oxidases are further described in at least Birchfield, et al., Biochemistry, 37(19):6905-6910 (1998); Fingar, et al., Cancer Res., 57(20):4551-4556 (1997); Arnould, et al., Biochemistry, 36(33):10178-10184 (1997); and Dailey and Dailey, Cell Mol. Biol., 43(1):67-73 (1997).

#### 13. PRO1272

The cement gland is an ectodermal organ in the head of frog embryos, lying anterior to any neural tissue. The cement gland, like neural tissue, has been shown to be induced by the dorsal mesoderm. XAG-1 is a cement gland specific protein that is useful as a marker of cement gland induction during development. See, Sive, et al., Cell, 58(1):171-180 (1989); Itoh, et al., Development, 121(12):3979-3988 (1995). XAG-2 and other proteins related to the XAG family are further described in Aberger, et al., Mech. Dev., 72(1-2):115-130 (1998) and Gammill and Sive, Development, 124(2):471-481 (1997). Thus, novel polypeptides having sequence identity with XAG proteins are of interest.

#### 14. PRO1419

Efforts are being undertaken by both industry and academia to identify new, native secreted proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted proteins. We herein describe the identification and characterization of novel



secreted polypeptides, designated herein as PRO1419 polypeptides.

# 15. **PRO4999**

Uromodulin is synthesized in the kidney and is the most abundant protein in normal human urine. The amino acid sequence encoded by one of the exons of the uromodulin gene has homology to the low-density-lipoprotein receptor and the epidermal growth factor precursor. Pennica et al., Science 236:83-88 (1987). The function of uromodulin is not known; however, it may function as a unique renal regulatory glycoprotein that specifically binds to and regulates the circulating activity of a number of potent cytokines, as it binds to IL-1, IL-2 and TNF with high affinity. See Hession et al., Science 237:1479-1484 (1987). Su et al. suggest that uromodulin plays a significant role in the innate immunity of the urinary system and that the immunostimulatory activity of uromodulin is potentially useful for immunotherapy. Su et al., J. Immunology, 158:3449-3456 (1997).

We herein describe the identification and characterization of novel polypeptides having sequence similarity to uromodulin, designated herein as PRO4999 polypeptides.

# 16. **PRO7170**

Efforts are being undertaken by both industry and academia to identify new, native secreted proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted proteins. We herein describe the identification and characterization of novel secreted polypeptides, designated herein as PRO7170 polypeptides.

# 17. **PRO248**

Cytokines have been implicated in the pathogenesis of a number of brain diseases in which neurological dysfunction has been attributed to a change in amino acid neurotransmitter metabolism. In particular, members of the transforming growth factor $\beta$ s (TGF $\beta$ ) have been implicated. Transforming growth peptides are small polypeptides that were first identified by their ability to induce proliferation and transformation in noncancerous cells in culture. Although initially defined as a growth factor, TGF $\beta$  also inhibits proliferation of epithelial, endothelial, lymphoid, and hematopoietic cells. This cytokine is thought to play an important role in regulating the duration of the inflammatory response, allowing the healing process to proceed. It is also a potent immunomodulator, which has many pleiotrophic effects, including regulating many other cytokines.

The TGF $\beta$  family includes basic myelin proteins (BMP-2, BMP-4, BMP-5, BMP-6, BMP-7), activins A & B, decapentaplegic (dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs) 1, 3, and 9, nodal, MIS, Inhibin  $\alpha$ , transforming growth factors betas (TGF- $\beta$ 1, TGF- $\beta$ 2, TGF- $\beta$ 3, TGF- $\beta$ 5), and glial-derived neurotrophic factor (GDNF), Atrisano, *et al.*, J. Biochemica et Biophysica Acta, 1222:71-80 (1994). Of particular interest are the growth differentiation factors, for as their name implies, these factors are implicated in the differentiation of cells.

Thus, identifying proteins having homology to the TGF $\beta$  family members, particularly growth differentiation factor (GDF) 3, is of importance to the medical and industrial community. Generally, proteins having homology to each other have similar function. It is also of interest when proteins having homology do not have similar functions, indicating that certain structural motifs identify information other than function, such as locality of function.

## 18. PRO353

The complement proteins comprise a large group of serum proteins some of which act in an enzymatic cascade, producing effector molecules involved in inflammation. The complement proteins are of particular importance in regulating movement and function of cells involved in inflammation. Given the physiological importance of inflammation and related mechanisms *in vivo*, efforts are currently being under taken to identify new, native proteins which are involved in inflammation. We describe herein the identification and characterization of novel polypeptides which have homology to complement proteins, designated herein as PRO353 polypeptides.

## 19. PRO533

Growth factors are molecular signals or mediators that enhance cell growth or proliferation, alone or in concert, by binding to specific cell surface receptors. however, there are other cellular reactions than only growth upon expression to growth factors. As a result, growth factors are better characterized as multifunctional and potent cellular regulators. Their biological effects include proliferation, chemotaxis and stimulation of extracellular matrix production. Growth factors can have both stimulatory and inhibitory effects. For example, transforming growth factors (TGF- $\beta$ ) is highly pleiotropic and can stimulate proliferation in some cells, especially connective tissues, while being a potent inhibitor of proliferation in others, such as lymphocytes and epithelial cells.

The physiological effect of growth stimulation or inhibition by growth factors depends upon the state of development and differentiation of the target tissue. The mechanism of local cellular regulation by classical endocrine molecules comprehends autocrine (same cell), juxtacrine (neighbor cell), and paracrine (adjacent cell) pathways. Peptide growth factors are elements of a complex biological language, providing the basis for intercellular communication. They permit cells to convey information between each other, mediate interaction between cells and change gene expression. the effect of these multifunctional and pluripotent factors is dependent on the presence or absence of other peptides.

Fibroblast growth factors (FGFs) are a family of heparin-binding, potent mitogens for both normal diploid fibroblasts and established cell lines, Godpodarowicz, D. et al. (1984), Proc. Natl. Acad. Sci. USA 81: 6983. the FGF family comprises acidic FGF (FGF-1), basic FGF (FGF-2), INT-2 (FGF-3), K-FGF/HST (FGF-4), FGF-5, FGF-6, KGF (FGF-7), AIGF (FGF-8) among others. All FGFs have two conserved cysteine residues and share 30-50% sequence homology at the amino acid level. These factors are mitogenic for a wide variety of normal diploid mesoderm-derived and neural crest-derived cells, inducing granulosa cells, adrenal cortical cells, chondrocytes, myoblasts, corneal and vascular endothelial cells (bovine or human),

vascular smooth muscle cells, lens, retina and prostatic epithelial cells, oligodendrocytes, astrocytes, chondrocytes, myoblasts and osteoblasts.

Fibroblast growth factors can also stimulate a large number of cell types in a non-mitogenic manner. These activities include promotion of cell migration into a wound area (chemotaxis), initiation of new blood vessel formation (angiogenesis), modulation of nerve regeneration and survival (neurotrophism), modulation of endocrine functions, and stimulation or suppression of specific cellular protein expression, extracellular matrix production and cell survival. Baird, A. & Bohlen, P., *Handbook of Exp. Pharmacol.* 95(1): 369-418 (1990). These properties provide a basis for using fibroblast growth factors in therapeutic approaches to accelerate wound healing, nerve repair, collateral blood vessel formation, and the like. For example, fibroblast growth factors, have been suggested to minimize myocardium damage in heart disease and surgery (U.S.P. 4,378,437).

We herein describe the identification and characterization of novel polypeptides having homology to FGF, herein designated PRO533 polypeptides.

**20. PRO301**

The widespread occurrence of cancer has prompted the devotion of considerable resources and discovering new treatments of treatment. One particular method involves the creation of tumor or cancer specific monoclonal antibodies (mAbs) which are specific to tumor antigens. Such mAbs, which can distinguish between normal and cancerous cells are useful in the diagnosis, prognosis and treatment of the disease. Particular antigens are known to be associated with neoplastic diseases, such as colorectal cancer.

One particular antigen, the A33 antigen is expressed in more than 90% of primary or metastatic colon cancers as well as normal colon epithelium. Since colon cancer is a widespread disease, early diagnosis and treatment is an important medical goal. Diagnosis and treatment of colon cancer can be implemented using monoclonal antibodies (mAbs) specific therefore having fluorescent, nuclear magnetic or radioactive tags. Radioactive gene, toxins and/or drug tagged mAbs can be used for treatment *in situ* with minimal patient description. mAbs can also be used to diagnose during the diagnosis and treatment of colon cancers. For example, when the serum levels of the A33 antigen are elevated in a patient, a drop of the levels after surgery would indicate the tumor resection was successful. On the other hand, a subsequent rise in serum A33 antigen levels after surgery would indicate that metastases of the original tumor may have formed or that new primary tumors may have appeared. Such monoclonal antibodies can be used in lieu of, or in conjunction with surgery and/or other chemotherapies. For example, U.S.P. 4,579,827 and U.S.S.N. 424,991 (E.P. 199,141) are directed to therapeutic administration of monoclonal antibodies, the latter of which relates to the application of anti-A33 mAb.

Many cancers of epithelial origin have adenovirus receptors. In fact, adenovirus-derived vectors have been proposed as a means of inserting antisense nucleic acids into tumors (U.S.P. 5,518,885). Thus, the association of viral receptors with neoplastic tumors is not unexpected.

We herein describe the identification and characterization of novel polypeptides having homology to certain cancer-associated antigens, designated herein as PRO301 polypeptides.

## 21. **PRO187**

Growth factors are molecular signals or mediators that enhance cell growth or proliferation, alone or in concert, by binding to specific cell surface receptors. However, there are other cellular reactions than only growth upon expression to growth factors. As a result, growth factors are better characterized as multifunctional and potent cellular regulators. Their biological effects include proliferation, chemotaxis and stimulation of extracellular matrix production. Growth factors can have both stimulatory and inhibitory effects. For example, transforming growth factor (TGF- $\beta$ ) is highly pleiotropic and can stimulate proliferation in some cells, especially connective tissue, while being a potent inhibitor of proliferation in others, such as lymphocytes and epithelial cells.

The physiological effect of growth stimulation or inhibition by growth factors depends upon the state of development and differentiation of the target tissue. The mechanism of local cellular regulation by classical endocrine molecules involves comprehends autocrine (same cell), juxtacrine (neighbor cell), and paracrine (adjacent cells) pathways. Peptide growth factors are elements of a complex biological language, providing the basis for intercellular communication. They permit cells to convey information between each other, mediate interaction between cells and change gene expression. The effect of these multifunctional and pluripotent factors is dependent on the presence or absence of other peptides.

FGF-8 is a member of the fibroblast growth factors (FGFs) which are a family of heparin-binding, potent mitogens for both normal diploid fibroblasts and established cell lines, Gospodarowicz *et al.* (1984), *Proc. Natl. Acad. Sci. USA* **81**:6963. The FGF family comprises acidic FGF (FGF-1), basic FGF (FGF-2), INT-2 (FGF-3), K-FGF/HST (FGF-4), FGF-5, FGF-6, KGF (FGF-7), AIGF (FGF-8) among others. All FGFs have two conserved cysteine residues and share 30-50% sequence homology at the amino acid level. These factors are mitogenic for a wide variety of normal diploid mesoderm-derived and neural crest-derived cells, including granulosa cells, adrenal cortical cells, chondrocytes, myoblasts, corneal and vascular endothelial cells (bovine or human), vascular smooth muscle cells, lens, retina and prostatic epithelial cells, oligodendrocytes, astrocytes, chondrocytes, myoblasts and osteoblasts.

Fibroblast growth factors can also stimulate a large number of cell types in a non-mitogenic manner. These activities include promotion of cell migration into wound area (chemotaxis), initiation of new blood vessel formation (angiogenesis), modulation of nerve regeneration and survival (neurotrophism), modulation of endocrine functions, and stimulation or suppression of specific cellular protein expression, extracellular matrix production and cell survival. Baird & Bohlen, *Handbook of Exp. Pharmacol.* **95**(1): 369-418, Springer, (1990). These properties provide a basis for using fibroblast growth factors in therapeutic approaches to accelerate wound healing, nerve repair, collateral blood vessel formation, and the like. For example, fibroblast growth factors have been suggested to minimize myocardium damage in heart disease and surgery (U.S.P. 4,378,347).

FGF-8, also known as androgen-induced growth factor (AIGF), is a 215 amino acid protein which shares 30-40% sequence homology with the other members of the FGF family. FGF-8 has been proposed to be under androgenic regulation and induction in the mouse mammary carcinoma cell line SC3. Tanaka *et al.*, *Proc. Natl. Acad. Sci. USA* **89**: 8928-8932 (1992); Sato *et al.*, *J. Steroid Biochem. Molec. Biol.* **47**: 91-98

(1993). As a result, FGF-8 may have a local role in the prostate, which is known to be an androgen-responsive organ. FGF-8 can also be oncogenic, as it displays transforming activity when transfected into NIH-3T3 fibroblasts. Kouhara *et al.*, *Oncogene* 9 455-462 (1994). While FGF-8 has been detected in heart, brain, lung, kidney, testis, prostate and ovary, expression was also detected in the absence of exogenous androgens. Schmitt *et al.*, *J. Steroid Biochem. Mol. Biol.* 57 (3-4): 173-78 (1996).

5 FGF-8 shares the property with several other FGFs of being expressed at a variety of stages of murine embryogenesis, which supports the theory that the various FGFs have multiple and perhaps coordinated roles in differentiation and embryogenesis. Moreover, FGF-8 has also been identified as a protooncogene that cooperates with Wnt-1 in the process of mammary tumorigenesis (Shackleford *et al.*, *Proc. Natl. Acad. Sci. USA* 90, 740-744 (1993); Heikinheimo *et al.*, *Mech. Dev.* 48: 129-138 (1994)).

10 In contrast to the other FGFs, FGF-8 exists as three protein isoforms, as a result of alternative splicing of the primary transcript. Tanaka *et al.*, *supra*. Normal adult expression of FGF-8 is weak and confined to gonadal tissue, however northern blot analysis has indicated that FGF-8 mRNA is present from day 10 through day 12 of murine gestation, which suggests that FGF-8 is important to normal development. Heikinheimo *et al.*, *Mech Dev.* 48(2): 129-38 (1994). Further *in situ* hybridization assays between day 8 and 16 of gestation  
15 indicated initial expression in the surface ectoderm of the first bronchial arches, the frontonasal process, the forebrain and the midbrain-hindbrain junction. At days 10-12, FGF-8 was expressed in the surface ectoderm of the forelimb and hindlimb buds, the nasal pits and nasopharynx, the infundibulum and in the telencephalon, diencephalon and metencephalon. Expression continues in the developing hindlimbs through day 13 of gestation, but is undetectable thereafter. The results suggest that FGF-8 has a unique temporal and spatial  
20 pattern in embryogenesis and suggests a role for this growth factor in multiple regions of ectodermal differentiation in the post-gastrulation embryo.

We herein describe the identification of novel polypeptides having homology to FGF-8, wherein those polypeptides are herein designated PRO187 polypeptides.

## 25 22. PRO337

Neuronal development in higher vertebrates is characterized by processes that must successfully navigate distinct cellular environment en route to their synaptic targets. The result is a functionally precise formation of neural circuits. The precision is believed to result from mechanisms that regulate growth cone pathfinding and target recognition, followed by later refinement and remodeling of such projections by events  
30 that require neuronal activity, Goodman and Shatz, *Cell/Neuron* [Suppl.] 72(10): 77-98 (1993). It is further evident that different neurons extend nerve fibers that are biochemically distinct and rely on specific guidance cues provided by cell-cell, cell-matrix, and chemotrophic interactions to reach their appropriate synaptic targets, Goodman *et al.*, *supra*.

One particular means by which diversity of the neuronal cell surface may be generated is through  
35 differential expression of cell surface proteins referred to as cell adhesion molecules (CAMs). Neuronally expressed CAMs have been implicated in diverse developmental processes, including migration of neurons along radial glial cells, providing permissive or repulsive substrates for neurite extension, and in promoting

the selective fasciculation of axons in projectional pathways. Jessel, *Neuron* 1: 3-13 (1988); Edelman and Crossin, *Annu. Rev. Biochem.* 60: 155-190 (1991). Interactions between CAMs present on the growth cone membrane and molecules on opposing cell membranes or in the extracellular matrix are thought to provide the specific guidance cues that direct nerve fiber outgrowth along appropriate projectional pathways. Such interactions are likely to result in the activation of various second messenger systems within the growth cone that regulate neurite outgrowth. Doherty and Walsh, *Curr. Opin. Neurobiol.* 2: 595-601 (1992).

In higher vertebrates, most neural CAMs have been found to be members of three major structural families of proteins: the integrins, the cadherins, and the immunoglobulin gene superfamily (IgSF). Jessel, *supra.*; Takeichi, *Annu. Rev. Biochem.* 59: 237-252 (1990); Reichardt and Tomaselli, *Annu. Rev. Neurosci.* 14: 531-570 (1991). Cell adhesion molecules of the IgSF (or Ig-CAMs), in particular, constitute a large family of proteins frequently implicated in neural cell interactions and nerve fiber outgrowth during development, Salzer and Colman, *Dev. Neurosci.* 11: 377-390 (1989); Brummendorf and Rathjen, *J. Neurochem.* 61: 1207-1219 (1993). However, the majority of mammalian Ig-CAMs appear to be too widely expressed to specify navigational pathways or synaptic targets suggesting that other CAMs, yet to be identified, have role in these more selective interactions of neurons.

Many of the known neural Ig-CAMs have been found to be attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor. Additionally, many studies have implicated GPI-anchored proteins in providing specific guidance cues during the outgrowth on neurons in specific pathways. In studies of the grasshopper nervous system, treatment of embryos with phosphatidylinositol-specific phospholipase C (PIPLC), which selectively removes GPI-anchored proteins from the surfaces of cells, resulted in misdirection and faulty navigation among subsets of pioneering growth cones, as well as inhibited migratory patterns of a subset of early neurons, Chang et al., *Devel.* 114: 507-519 (1992). The projection of retinal fibers to the optic tectum appears to depend, in part, on a 33 kDa GPI-anchored protein, however, the precise nature of this protein is unknown. Stahl et al., *Neuron* 5: 735-743 (1990).

The expression of various GPI-anchored proteins has been characterized amongst the different populations of primary rat neurons amongst dorsal root ganglion, sympathetic neurons of the cervical ganglion, sympathetic neurons of the superior cervical ganglion, and cerebellar granule neurons. Rosen et al., *J. Cell Biol.* 117: 617-627 (1992). In contrast to the similar pattern of total membrane protein expression by these different types of neurons, striking differences were observed in the expression of GPI-anchored proteins between these neurons. Recently, a 65 kDa protein band known as neurotrimin was discovered and found to be differentially expressed by primary neurons (Rosen et al., *supra.*), and restricted to the nervous system and found to be the most abundant and earliest expressed of the GPI-anchored species in the CNS. Struyk et al., *J. Neuroscience* 15(3): 2141-2156 (1995). The discovery of neurotrimin has further lead to the identification of a family of IgSF members, each containing three Ig-like domains that share significant amino acid identity, now termed IgLON. Struyk et al., *supra.*; Pimenta et al., *Gene* 170(2): 189-95 (1996).

Additional members of the IgLON subfamily include opiate binding cell adhesion molecule (OBCAM), Schofield et al., *EMBO J.* 8: 489-495 (1989); limbic associated membrane protein (LAMP), Pimenta et al., *supra.*; CEPU-1; GP55, Wilson et al., *J. Cell Sci.* 109: 3129-3138 (1996); *Eur. J. Neurosci.* 9(2): 334-41

(1997); and AvGp50, Hancox et al., *Brain Res. Mol. Brain Res.* 44(2): 273-85 (1997).

While the expression of neurotrimin appears to be widespread, it does appear to correlated with the development of several neural circuits. For example, between E18 and P10, neurotrimin mRNA expression within the forebrain is maintained at high levels in neurons of the developing thalamus, cortical subplate, and cortex, particularly laminae V and VI (with less intense expression in II, II, and IV, and minimal expression in lamina I). Cortical subplate neurons may provide an early, temporary scaffold for the ingrowing thalamic afferents en route to their final synaptic targets in the cortex. Allendoerfer and Shatz, *Annu. Rev. Neurosci.* 17: 185-218 (1994). Conversely, subplate neurons have been suggested to be required for cortical neurons from layer V to select VI to grow into the thalamus, and neurons from layer V to select their targets in the colliculus, pons, and spinal cord (McConnell et al., *J. Neurosci.* 14: 1892-1907 (1994). The high level expression of neurotrimin in many of these projections suggests that it could be involved in their development.

In the hindbrain, high levels of neurotrimin message expression were observed within the pontine nucleus and by the internal granule cells and Purkinje cells of the cerebellum. The pontine nucleus received afferent input from a variety of sources including corticopontine fibers of layer V, and is a major source of afferent input, via mossy fibers, to the granule cells which, in turn, are a major source of afferent input via parallel fibers to Purkinje cells. [Palay and Chan-Palay, *The cerebellar cortex: cytology and organization.* New York: Springer (1974)]. High level expression of neurotrimin these neurons again suggests potential involvement in the establishment of these circuits.

Neurotrimin also exhibits a graded expression pattern in the early postnatal striatum. Increased neurotrimin expression is found overlying the dorsolateral striatum of the rat, while lesser hybridization intensity is seen overlying the ventromedial striatum. Struyk et al., *supra*. This region of higher neurotrimin hybridization intensity does not correspond to a cytoarchitecturally differentiable region, rather it corresponds to the primary area of afferent input from layer VI of the contralateral sensorimotor cortex (Gerfen, *Nature* 311: 461-464 (1984); Donoghue and Herkenham, *Brain Res.* 365: 397-403 (1986)). The ventromedial striatum, by contrast, receives the majority of its afferent input from the perirhinal and association cortex. It is noteworthy that a complementary graded pattern of LAMP expression, has been observed within the striatum, with highest expression in ventromedial regions, and lowest expression dorsolaterally. Levitt, *Science* 223: 299-301 (1985); Chesselet et al., *Neuroscience* 40: 725-733 (1991).

### 23. **PRO1411**

Efforts are being undertaken by both industry and academia to identify new, native secreted proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted proteins. We herein describe the identification and characterization of a novel secreted protein designated herein as PRO1411.

### 24. **PRO4356**

Glycosylphosphatidylinositol (GPI) anchored proteoglycans are generally localized to the cell surface and are thus known to be involved in the regulation of responses of cells to numerous growth factors, cell

adhesion molecules and extracellular matrix components. The metastasis-associated GPI-anchored protein (MAGPIAP) is one of these cell surface proteins which appears to be involved in metastasis. Metastasis is the form of cancer wherein the transformed or malignant cells are traveling and spreading the cancer from one site to another. Therefore, identifying the polypeptides related to metastasis and MAGPIAP is of interest.

5      25.      **PRO246**

The cell surface protein HCAR is a membrane-bound protein that acts as a receptor for subgroup C of the adenoviruses and subgroup B of the coxsackieviruses. Thus, HCAR may provide a means for mediating viral infection of cells in that the presence of the HCAR receptor on the cellular surface provides a binding site for viral particles, thereby facilitating viral infection.

10            In light of the physiological importance of membrane-bound proteins and specifically those which serve a cell surface receptor for viruses, efforts are currently being undertaken by both industry and academia to identify new, native membrane-bound receptor proteins. Many of these efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel receptor proteins. We herein describe a novel membrane-bound polypeptide (designated herein as PRO246) having homology to the  
15            cell surface protein HCAR and to various tumor antigens including A33 and carcinoembryonic antigen, wherein this polypeptide may be a novel cell surface virus receptor or tumor antigen.

26.      **PRO265**

20            Protein-protein interactions include receptor and antigen complexes and signaling mechanisms. As more is known about the structural and functional mechanisms underlying protein-protein interactions, protein-protein interactions can be more easily manipulated to regulate the particular result of the protein-protein interaction. Thus, the underlying mechanisms of protein-protein interactions are of interest to the scientific and medical community.

25            All proteins containing leucine-rich repeats are thought to be involved in protein-protein interactions. Leucine-rich repeats are short sequence motifs present in a number of proteins with diverse functions and cellular locations. The crystal structure of ribonuclease inhibitor protein has revealed that leucine-rich repeats correspond to beta-alpha structural units. These units are arranged so that they form a parallel beta-sheet with one surface exposed to solvent, so that the protein acquires an unusual, nonglobular shape. These two features have been indicated as responsible for the protein-binding functions of proteins containing leucine-rich repeats.  
30            See, Kobe and Deisenhofer, Trends Biochem. Sci., 19(10):415-421 (Oct. 1994).

A study has been reported on leucine-rich proteoglycans which serve as tissue organizers, orienting and ordering collagen fibrils during ontogeny and are involved in pathological processes such as wound healing, tissue repair, and tumor stroma formation. Iozzo, R. V., Crit. Rev. Biochem. Mol. Biol., 32(2):141-174 (1997). Others studies implicating leucine rich proteins in wound healing and tissue repair are De La Salle, C., et al., Vouv. Rev. Fr. Hematol. (Germany), 37(4):215-222 (1995), reporting mutations in the leucine rich motif in a complex associated with the bleeding disorder Bernard-Soulier syndrome and Chlemetson, K. J., Thromb. Haemost. (Germany), 74(1):111-116 (July 1995), reporting that platelets have  
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leucine rich repeats. Another protein of particular interest which has been reported to have leucine-rich repeats is the SLIT protein which has been reported to be useful in treating neuro-degenerative diseases such as Alzheimer's disease, nerve damage such as in Parkinson's disease, and for diagnosis of cancer, see, Artavanistsakonas, S. and Rothberg, J. M., WO9210518-A1 by Yale University. Other studies reporting on the biological functions of proteins having leucine-rich repeats include: Tayar, N., et al., Mol. Cell  
5 Endocrinol., (Ireland), 125(1-2):65-70 (Dec. 1996) (gonadotropin receptor involvement); Miura, Y., et al., Nippon Rinsho (Japan), 54(7):1784-1789 (July 1996) (apoptosis involvement); Harris, P. C., et al., J. Am. Soc. Nephrol., 6(4):1125-1133 (Oct. 1995) (kidney disease involvement); and Ruoslahti, E. I., et al., WO9110727-A by La Jolla Cancer Research Foundation (decorin binding to transforming growth factor- $\beta$  involvement for treatment for cancer, wound healing and scarring). Also of particular interest is fibromodulin  
10 and its use to prevent or reduce dermal scarring. A study of fibromodulin is found in U.S. Patent No. 5,654,270 to Ruoslahti, et al.

Efforts are therefore being undertaken by both industry and academia to identify new proteins having leucine rich repeats to better understand protein-protein interactions. Of particular interest are those proteins having leucine rich repeats and homology to known proteins having leucine rich repeats such as fibromodulin,  
15 the SLIT protein and platelet glycoprotein V. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted and membrane-bound proteins having leucine rich repeats. We herein describe the identification and characterization of novel polypeptides having homology to fibromodulin, herein designated as PRO265 polypeptides.

## 20 27. PRO941

Cadherins are a large family of transmembrane proteins. Cadherins comprise a family of calcium-dependent glycoproteins that function in mediating cell-cell adhesion in virtually all solid tissues of multicellular organisms. At least cadherins 1-13 as well as types B, E, EP, M, N, P and R have been identified and characterized. Among the functions cadherins are known for, with some exceptions, are that cadherins  
25 participate in cell aggregation and are associated with cell-cell adhesion sites. Recently, it has been reported that while all cadherins share multiple repeats of a cadherin specific motif believed to correspond to folding of extracellular domains, members of the cadherin superfamily have divergent structures and, possibly, functions. In particular it has been reported that members of the cadherin superfamily are involved in signal transduction. See, Suzuki, J. Cell Biochem., 61(4):531-542 (1996). Cadherins are further described in  
30 Tanihara et al., J. Cell Sci., 107(6):1697-1704 (1994), Aberle et al., J. Cell Biochem., 61(4):514-523 (1996) and Tanihara et al., Cell Adhes. Commun., 2(1):15-26 (1994). We herein describe the identification and characterization of a novel polypeptide having homology to a cadherin protein, designated herein as PRO941.

## 35 28. PRO10096

Interleukin-10 (IL-10) is a pleiotropic immunosuppressive cytokine that has been implicated as an important regulator of the functions of myeloid and lymphoid cells. It has been demonstrated that IL-10 functions as a potent inhibitor of the activation of the synthesis of various inflammatory cytokines including,

for example, IL-1, IL-6, IFN- $\gamma$  and TNF- $\alpha$  (Gesser et al., Proc. Natl. Acad. Sci. USA 94:14620-14625 (1997)). Moreover, IL-10 has been demonstrated to strongly inhibit several of the accessory activities of macrophages, thereby functioning as a potent suppressor of the effector functions of macrophages, T-cells and NK cells (Kuhn et al., Cell 75:263-274 (1993)). Furthermore, IL-10 has been strongly implicated in the regulation of B-cell, mast cell and thymocyte differentiation.

IL-10 was independently identified in two separate lines of experiments. First, cDNA clones encoding murine IL-10 were identified based upon the expression of cytokine synthesis inhibitory factor (Moore et al., Science 248:1230-1234 (1990)), wherein the human IL-10 counterpart cDNAs were subsequently identified by cross-hybridization with the murine IL-10 cDNA (Viera et al., Proc. Natl. Acad. Sci. USA 88:1172-1176 (1991)). Additionally, IL-10 was independently identified as a B-cell-derived mediator which functioned to co-stimulate active thymocytes (Suda et al., Cell Immunol. 129:228 (1990)).

We herein describe the identification and characterization of novel polypeptides having sequence similarity to IL-10, designated herein as PRO10096 polypeptides.

## 29. **PRO6003**

Efforts are being undertaken by both industry and academia to identify new, native receptor or membrane-bound proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel receptor or membrane-bound proteins. We herein describe the identification and characterization of novel polypeptides designated herein as PRO6003 polypeptides.

## 20 **SUMMARY OF THE INVENTION**

In one embodiments of the present invention, the invention provides vectors comprising DNA encoding any of the herein described polypeptides. Host cell comprising any such vector are also provided. By way of example, the host cells may be CHO cells, *E. coli*, or yeast. A process for producing any of the herein described polypeptides is further provided and comprises culturing host cells under conditions suitable for expression of the desired polypeptide and recovering the desired polypeptide from the cell culture.

In other embodiments, the invention provides chimeric molecules comprising any of the herein described polypeptides fused to a heterologous polypeptide or amino acid sequence. Example of such chimeric molecules comprise any of the herein described polypeptides fused to an epitope tag sequence or a Fc region of an immunoglobulin.

In another embodiment, the invention provides an antibody which specifically binds to any of the above or below described polypeptides. Optionally, the antibody is a monoclonal antibody, humanized antibody, antibody fragment or single-chain antibody.

In yet other embodiments, the invention provides oligonucleotide probes useful for isolating genomic and cDNA nucleotide sequences or as antisense probes, wherein those probes may be derived from any of the above or below described nucleotide sequences.

In other embodiments, the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence that encodes a PRO polypeptide.

In one aspect, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule encoding a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of the full-length amino acid sequence as disclosed herein, or (b) the complement of the DNA molecule of (a).

In other aspects, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule comprising the coding sequence of a full-length PRO polypeptide cDNA as disclosed herein, the coding sequence of a PRO polypeptide lacking the signal peptide as disclosed herein, the coding sequence of an extracellular domain of a transmembrane PRO polypeptide, with or without the signal peptide, as disclosed herein or the coding sequence of any other specifically defined fragment of the full-length amino acid sequence as disclosed herein, or (b) the complement of the DNA molecule of (a).

In a further aspect, the invention concerns an isolated nucleic acid molecule comprising a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81 % nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about

83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule that encodes the same mature polypeptide encoded by any of the human protein cDNAs deposited with the ATCC as disclosed herein, or (b) the complement of the DNA molecule of (a).

Another aspect the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a PRO polypeptide which is either transmembrane domain-deleted or transmembrane domain-inactivated, or is complementary to such encoding nucleotide sequence, wherein the transmembrane domain(s) of such polypeptide are disclosed herein. Therefore, soluble extracellular domains of the herein described PRO polypeptides are contemplated.

Another embodiment is directed to fragments of a PRO polypeptide coding sequence, or the complement thereof, that may find use as, for example, hybridization probes, for encoding fragments of a PRO polypeptide that may optionally encode a polypeptide comprising a binding site for an anti-PRO antibody or as antisense oligonucleotide probes. Such nucleic acid fragments are usually at least about 20 nucleotides in length, alternatively at least about 30 nucleotides in length, alternatively at least about 40 nucleotides in length, alternatively at least about 50 nucleotides in length, alternatively at least about 60 nucleotides in length, alternatively at least about 70 nucleotides in length, alternatively at least about 80 nucleotides in length, alternatively at least about 90 nucleotides in length, alternatively at least about 100 nucleotides in length, alternatively at least about 110 nucleotides in length, alternatively at least about 120 nucleotides in length, alternatively at least about 130 nucleotides in length, alternatively at least about 140 nucleotides in length, alternatively at least about 150 nucleotides in length, alternatively at least about 160 nucleotides in length, alternatively at least about 170 nucleotides in length, alternatively at least about 180 nucleotides in length, alternatively at least about 190 nucleotides in length, alternatively at least about 200 nucleotides in length, alternatively at least about 250 nucleotides in length, alternatively at least about 300 nucleotides in length, alternatively at least about 350 nucleotides in length, alternatively at least about 400 nucleotides in length, alternatively at least about 450 nucleotides in length, alternatively at least about 500 nucleotides in length, alternatively at least about 600 nucleotides in length, alternatively at least about 700 nucleotides in length, alternatively at least about 800 nucleotides in length, alternatively at least about 900 nucleotides in length and alternatively at least about 1000 nucleotides in length, wherein in this context the term "about" means the referenced nucleotide sequence length plus or minus 10% of that referenced length. It is noted that novel fragments of a PRO polypeptide-encoding nucleotide sequence may be determined in a routine manner by

aligning the PRO polypeptide-encoding nucleotide sequence with other known nucleotide sequences using any of a number of well known sequence alignment programs and determining which PRO polypeptide-encoding nucleotide sequence fragment(s) are novel. All of such PRO polypeptide-encoding nucleotide sequences are contemplated herein. Also contemplated are the PRO polypeptide fragments encoded by these nucleotide molecule fragments, preferably those PRO polypeptide fragments that comprise a binding site for an anti-PRO antibody.

In another embodiment, the invention provides isolated PRO polypeptide encoded by any of the isolated nucleic acid sequences hereinabove identified.

In a certain aspect, the invention concerns an isolated PRO polypeptide, comprising an amino acid sequence having at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of the full-length amino acid sequence as disclosed herein.

In a further aspect, the invention concerns an isolated PRO polypeptide comprising an amino acid sequence having at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to an amino acid sequence encoded by any of the human protein cDNAs deposited with the ATCC as disclosed herein.

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## **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 shows a nucleotide sequence (SEQ ID NO:3) of a native sequence PRO196 cDNA, wherein SEQ ID NO:3 is a clone designated herein as "DNA22779-1130".

Figure 2 shows the amino acid sequence (SEQ ID NO:4) derived from the coding sequence of SEQ ID NO:3 shown in Figure 1.

5        Figure 3 shows a nucleotide sequence (SEQ ID NO:8) of a native sequence PRO444 cDNA, wherein SEQ ID NO:8 is a clone designated herein as "DNA26846-1397".

Figure 4 shows the amino acid sequence (SEQ ID NO:9) derived from the coding sequence of SEQ ID NO:8 shown in Figure 3.

10       Figure 5 shows a nucleotide sequence (SEQ ID NO:10) of a native sequence PRO183 cDNA, wherein SEQ ID NO:10 is a clone designated herein as "DNA28498".

Figure 6 shows the amino acid sequence (SEQ ID NO:11) derived from the coding sequence of SEQ ID NO:10 shown in Figure 5.

Figure 7 shows a nucleotide sequence (SEQ ID NO:12) of a native sequence PRO185 cDNA, wherein SEQ ID NO:12 is a clone designated herein as "DNA28503".

15       Figure 8 shows the amino acid sequence (SEQ ID NO:13) derived from the coding sequence of SEQ ID NO:12 shown in Figure 7.

Figure 9 shows a nucleotide sequence (SEQ ID NO:14) of a native sequence PRO210 cDNA, wherein SEQ ID NO:14 is a clone designated herein as "DNA32279-1131".

20       Figure 10 shows the amino acid sequence (SEQ ID NO:15) derived from the coding sequence of SEQ ID NO:14 shown in Figure 9.

Figure 11 shows a nucleotide sequence (SEQ ID NO:16) of a native sequence PRO215 cDNA, wherein SEQ ID NO:16 is a clone designated herein as "DNA32288-1132".

Figure 12 shows the amino acid sequence (SEQ ID NO:17) derived from the coding sequence of SEQ ID NO:16 shown in Figure 11.

25       Figure 13 shows a nucleotide sequence (SEQ ID NO:21) of a native sequence PRO217 cDNA, wherein SEQ ID NO:21 is a clone designated herein as "DNA33094-1131".

Figure 14 shows the amino acid sequence (SEQ ID NO:22) derived from the coding sequence of SEQ ID NO:21 shown in Figure 13.

30       Figure 15 shows a nucleotide sequence (SEQ ID NO:23) of a native sequence PRO242 cDNA, wherein SEQ ID NO:23 is a clone designated herein as "DNA33785-1143".

Figure 16 shows the amino acid sequence (SEQ ID NO:24) derived from the coding sequence of SEQ ID NO:23 shown in Figure 15.

Figure 17 shows a nucleotide sequence (SEQ ID NO:28) of a native sequence PRO288 cDNA, wherein SEQ ID NO:28 is a clone designated herein as "DNA35663-1129".

35       Figure 18 shows the amino acid sequence (SEQ ID NO:29) derived from the coding sequence of SEQ ID NO:28 shown in Figure 17.

Figure 19 shows a nucleotide sequence (SEQ ID NO:31) of a native sequence PRO365 cDNA, wherein SEQ ID NO:31 is a clone designated herein as "DNA46777-1253".

Figure 20 shows the amino acid sequence (SEQ ID NO:32) derived from the coding sequence of SEQ ID NO:31 shown in Figure 19.

5 Figure 21 shows a nucleotide sequence (SEQ ID NO:38) of a native sequence PRO1361 cDNA, wherein SEQ ID NO:38 is a clone designated herein as "DNA60783-1611".

Figure 22 shows the amino acid sequence (SEQ ID NO:39) derived from the coding sequence of SEQ ID NO:38 shown in Figure 21.

Figure 23 shows a nucleotide sequence (SEQ ID NO:40) of a native sequence PRO1308 cDNA, wherein SEQ ID NO:40 is a clone designated herein as "DNA62306-1570".

10 Figure 24 shows the amino acid sequence (SEQ ID NO:41) derived from the coding sequence of SEQ ID NO:40 shown in Figure 23.

Figure 25 shows a nucleotide sequence (SEQ ID NO:51) of a native sequence PRO1183 cDNA, wherein SEQ ID NO:51 is a clone designated herein as "DNA62880-1513".

15 Figure 26 shows the amino acid sequence (SEQ ID NO:52) derived from the coding sequence of SEQ ID NO:51 shown in Figure 25.

Figure 27 shows a nucleotide sequence (SEQ ID NO:53) of a native sequence PRO1272 cDNA, wherein SEQ ID NO:53 is a clone designated herein as "DNA64896-1539".

Figure 28 shows the amino acid sequence (SEQ ID NO:54) derived from the coding sequence of SEQ ID NO:53 shown in Figure 27.

20 Figure 29 shows a nucleotide sequence (SEQ ID NO:55) of a native sequence PRO1419 cDNA, wherein SEQ ID NO:55 is a clone designated herein as "DNA71290-1630".

Figure 30 shows the amino acid sequence (SEQ ID NO:56) derived from the coding sequence of SEQ ID NO:55 shown in Figure 29.

25 Figure 31 shows a nucleotide sequence (SEQ ID NO:57) of a native sequence PRO4999 cDNA, wherein SEQ ID NO:57 is a clone designated herein as "DNA96031-2664".

Figure 32 shows the amino acid sequence (SEQ ID NO:58) derived from the coding sequence of SEQ ID NO:57 shown in Figure 31.

Figure 33 shows a nucleotide sequence (SEQ ID NO:62) of a native sequence PRO7170 cDNA, wherein SEQ ID NO:62 is a clone designated herein as "DNA108722-2743".

30 Figure 34 shows the amino acid sequence (SEQ ID NO:63) derived from the coding sequence of SEQ ID NO:62 shown in Figure 33.

Figure 35 shows a nucleotide sequence (SEQ ID NO:64) of a native sequence PRO248 cDNA, wherein SEQ ID NO:64 is a clone designated herein as "DNA35674-1142".

35 Figure 36 shows the amino acid sequence (SEQ ID NO:65) derived from the coding sequence of SEQ ID NO:64 shown in Figure 35.

Figure 37 shows a nucleotide sequence (SEQ ID NO:72) of a native sequence PRO353 cDNA, wherein SEQ ID NO:72 is a clone designated herein as "DNA41234".



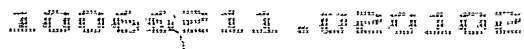


Figure 38 shows the amino acid sequence (SEQ ID NO:73) derived from the coding sequence of SEQ ID NO:72 shown in Figure 37.

Figure 39 shows a nucleotide sequence (SEQ ID NO:77) of a native sequence PRO1318 cDNA, wherein SEQ ID NO:77 is a clone designated herein as "DNA73838-1674".

5 Figure 40 shows the amino acid sequence (SEQ ID NO:78) derived from the coding sequence of SEQ ID NO:77 shown in Figure 39.

Figure 41 shows a nucleotide sequence (SEQ ID NO:79) of a native sequence PRO1600 cDNA, wherein SEQ ID NO:79 is a clone designated herein as "DNA77503-1686".

Figure 42 shows the amino acid sequence (SEQ ID NO:80) derived from the coding sequence of SEQ ID NO:79 shown in Figure 41.

10 Figure 43 shows a nucleotide sequence (SEQ ID NO:83) of a native sequence PRO9940 cDNA, wherein SEQ ID NO:83 is a clone designated herein as "DNA92282".

Figure 44 shows the amino acid sequence (SEQ ID NO:84) derived from the coding sequence of SEQ ID NO:83 shown in Figure 43.

15 Figure 45 shows a nucleotide sequence (SEQ ID NO:85) of a native sequence PRO533 cDNA, wherein SEQ ID NO:85 is a clone designated herein as "DNA49435-1219".

Figure 46 shows the amino acid sequence (SEQ ID NO:86) derived from the coding sequence of SEQ ID NO:85 shown in Figure 45.

Figure 47 shows a nucleotide sequence (SEQ ID NO:90) of a native sequence PRO301 cDNA, wherein SEQ ID NO:90 is a clone designated herein as "DNA40628-1216".

20 Figure 48 shows the amino acid sequence (SEQ ID NO:91) derived from the coding sequence of SEQ ID NO:90 shown in Figure 47.

Figure 49 shows a nucleotide sequence (SEQ ID NO:98) of a native sequence PRO187 cDNA, wherein SEQ ID NO:98 is a clone designated herein as "DNA27864-1155".

25 Figure 50 shows the amino acid sequence (SEQ ID NO:99) derived from the coding sequence of SEQ ID NO:98 shown in Figure 49.

Figure 51 shows a nucleotide sequence (SEQ ID NO:103) of a native sequence PRO337 cDNA, wherein SEQ ID NO:103 is a clone designated herein as "DNA43316-1237".

Figure 52 shows the amino acid sequence (SEQ ID NO:104) derived from the coding sequence of SEQ ID NO:103 shown in Figure 51.

30 Figure 53 shows a nucleotide sequence (SEQ ID NO:105) of a native sequence PRO1411 cDNA, wherein SEQ ID NO:105 is a clone designated herein as "DNA59212-1627".

Figure 54 shows the amino acid sequence (SEQ ID NO:106) derived from the coding sequence of SEQ ID NO:105 shown in Figure-53.

35 Figure 55 shows a nucleotide sequence (SEQ ID NO:107) of a native sequence PRO4356 cDNA, wherein SEQ ID NO:107 is a clone designated herein as "DNA86576-2595".

Figure 56 shows the amino acid sequence (SEQ ID NO:108) derived from the coding sequence of SEQ ID NO:107 shown in Figure 55.

Figure 57 shows a nucleotide sequence (SEQ ID NO:109) of a native sequence PRO246 cDNA, wherein SEQ ID NO:109 is a clone designated herein as "DNA35639-1172".

Figure 58 shows the amino acid sequence (SEQ ID NO:110) derived from the coding sequence of SEQ ID NO:109 shown in Figure 57.

5 Figure 59 shows a nucleotide sequence (SEQ ID NO:114) of a native sequence PRO265 cDNA, wherein SEQ ID NO:114 is a clone designated herein as "DNA36350-1158".

Figure 60 shows the amino acid sequence (SEQ ID NO:115) derived from the coding sequence of SEQ ID NO:114 shown in Figure 59.

Figure 61 shows a nucleotide sequence (SEQ ID NO:120) of a native sequence PRO941 cDNA, wherein SEQ ID NO:120 is a clone designated herein as "DNA53906-1368".

10 Figure 62 shows the amino acid sequence (SEQ ID NO:121) derived from the coding sequence of SEQ ID NO:120 shown in Figure 61.

Figure 63 shows a nucleotide sequence (SEQ ID NO:125) of a native sequence PRO10096 cDNA, wherein SEQ ID NO:125 is a clone designated herein as "DNA125185-2806".

15 Figure 64 shows the amino acid sequence (SEQ ID NO:126) derived from the coding sequence of SEQ ID NO:125 shown in Figure 63.

Figure 65 shows a nucleotide sequence (SEQ ID NO:127) of a native sequence PRO6003 cDNA, wherein SEQ ID NO:127 is a clone designated herein as "DNA83568-2692".

Figure 66 shows the amino acid sequence (SEQ ID NO:128) derived from the coding sequence of SEQ ID NO:127 shown in Figure 65.

20 Figures 67A-B show a nucleotide sequence (SEQ ID NO:129) of a native sequence PRO6004 cDNA, wherein SEQ ID NO:129 is a clone designated herein as "DNA92259".

Figure 68 shows the amino acid sequence (SEQ ID NO:130) derived from the coding sequence of SEQ ID NO:129 shown in Figures 67A-B.

25 Figure 69 shows a nucleotide sequence (SEQ ID NO:131) of a native sequence PRO350 cDNA, wherein SEQ ID NO:131 is a clone designated herein as "DNA44175-1314".

Figure 70 shows the amino acid sequence (SEQ ID NO:132) derived from the coding sequence of SEQ ID NO:131 shown in Figure 69.

Figure 71 shows a nucleotide sequence (SEQ ID NO:136) of a native sequence PRO2630 cDNA, wherein SEQ ID NO:136 is a clone designated herein as "DNA83551".

30 Figure 72 shows the amino acid sequence (SEQ ID NO:137) derived from the coding sequence of SEQ ID NO:136 shown in Figure 71.

Figure 73 shows a nucleotide sequence (SEQ ID NO:138) of a native sequence PRO6309 cDNA, wherein SEQ ID NO:138 is a clone designated herein as "DNA116510".

35 Figure 74 shows the amino acid sequence (SEQ ID NO:139) derived from the coding sequence of SEQ ID NO:138 shown in Figure 73.

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them, are contemplated by the present invention.

The approximate location of the "signal peptides" of the various PRO polypeptides disclosed herein are shown in the present specification and/or the accompanying figures. It is noted, however, that the C-terminal boundary of a signal peptide may vary, but most likely by no more than about 5 amino acids on either side of the signal peptide C-terminal boundary as initially identified herein, wherein the C-terminal boundary of the signal peptide may be identified pursuant to criteria routinely employed in the art for identifying that type of amino acid sequence element (e.g., Nielsen et al., Prot. Eng. 10:1-6 (1997) and von Heinje et al., Nucl. Acids. Res. 14:4683-4690 (1986)). Moreover, it is also recognized that, in some cases, cleavage of a signal sequence from a secreted polypeptide is not entirely uniform, resulting in more than one secreted species. These mature polypeptides, where the signal peptide is cleaved within no more than about 5 amino acids on either side of the C-terminal boundary of the signal peptide as identified herein, and the polynucleotides encoding them, are contemplated by the present invention.

"PRO polypeptide variant" means an active PRO polypeptide as defined above or below having at least about 80% amino acid sequence identity with a full-length native sequence PRO polypeptide sequence as disclosed herein, a PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Such PRO polypeptide variants include, for instance, PRO polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the full-length native amino acid sequence. Ordinarily, a PRO polypeptide variant will have at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to a full-length native sequence PRO polypeptide sequence as disclosed herein, a PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of a full-length PRO polypeptide sequence as disclosed herein. Ordinarily, PRO variant polypeptides are at least about 10 amino acids in length, alternatively at least about 20 amino acids in length, alternatively at least about 30 amino acids in length, alternatively at least about 40 amino acids in length, alternatively at least about 50 amino acids in length, alternatively at least about 60 amino acids in length, alternatively at least about 70 amino acids in length, alternatively at least about 80 amino acids in length, alternatively at least about

90 amino acids in length, alternatively at least about 100 amino acids in length, alternatively at least about 150 amino acids in length, alternatively at least about 200 amino acids in length, alternatively at least about 300 amino acids in length, or more.

"Percent (%) amino acid sequence identity" with respect to the PRO polypeptide sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific PRO polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2, wherein the complete source code for the ALIGN-2 program is provided in Table 1 below. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code shown in Table 1 below has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in Table 1 below. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. As examples of % amino acid sequence identity calculations using this method, Tables 2 and 3 demonstrate how to calculate the % amino acid sequence identity of the amino acid sequence designated "Comparison Protein" to the amino acid sequence designated "PRO", wherein "PRO" represents the amino acid sequence of a hypothetical PRO polypeptide of interest, "Comparison Protein" represents the amino acid sequence of a polypeptide against which the "PRO" polypeptide of interest is being compared, and "X", "Y" and "Z" each represent different hypothetical amino acid residues.

Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program. However, % amino acid sequence identity values may also be obtained as described below by using the WU-BLAST-2 computer program (Altschul et al., Methods in Enzymology 266:460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, i.e., the adjustable parameters, are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11, and scoring matrix = BLOSUM62. When WU-BLAST-2 is employed, a % amino acid sequence identity value is determined by dividing (a) the number of matching identical amino acid residues between the amino acid sequence of the PRO polypeptide of interest having a sequence derived from the native PRO polypeptide and the comparison amino acid sequence of interest (i.e., the sequence against which the PRO polypeptide of interest is being compared which may be a PRO variant polypeptide) as determined by WU-BLAST-2 by (b) the total number of amino acid residues of the PRO polypeptide of interest. For example, in the statement "a polypeptide comprising an the amino acid sequence A which has or having at least 80% amino acid sequence identity to the amino acid sequence B", the amino acid sequence A is the comparison amino acid sequence of interest and the amino acid sequence B is the amino acid sequence of the PRO polypeptide of interest.

Percent amino acid sequence identity may also be determined using the sequence comparison program NCBI-BLAST2 (Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997)). The NCBI-BLAST2 sequence comparison program may be downloaded from <http://www.ncbi.nlm.nih.gov> or otherwise obtained from the National Institute of Health, Bethesda, MD. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

In situations where NCBI-BLAST2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program NCBI-BLAST2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A.

"PRO variant polynucleotide" or "PRO variant nucleic acid sequence" means a nucleic acid molecule which encodes an active PRO polypeptide as defined below and which has at least about 80% nucleic acid sequence identity with a nucleotide acid sequence encoding a full-length native sequence PRO polypeptide

sequence as disclosed herein, a full-length native sequence PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Ordinarily, a PRO variant polynucleotide will have at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity with a nucleic acid sequence encoding a full-length native sequence PRO polypeptide sequence as disclosed herein, a full-length native sequence PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal sequence, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Variants do not encompass the native nucleotide sequence.

Ordinarily, PRO variant polynucleotides are at least about 30 nucleotides in length, alternatively at least about 60 nucleotides in length, alternatively at least about 90 nucleotides in length, alternatively at least about 120 nucleotides in length, alternatively at least about 150 nucleotides in length, alternatively at least about 180 nucleotides in length, alternatively at least about 210 nucleotides in length, alternatively at least about 240 nucleotides in length, alternatively at least about 270 nucleotides in length, alternatively at least about 300 nucleotides in length, alternatively at least about 450 nucleotides in length, alternatively at least about 600 nucleotides in length, alternatively at least about 900 nucleotides in length, or more.

"Percent (%) nucleic acid sequence identity" with respect to PRO-encoding nucleic acid sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the PRO nucleic acid sequence of interest, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. For purposes herein, however, % nucleic acid sequence identity values are generated using the sequence comparison computer program ALIGN-2, wherein the complete source code for the ALIGN-2 program is provided in Table 1 below. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code shown in Table 1 below has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S.

Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in Table 1 below. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for nucleic acid sequence comparisons, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

$$100 \text{ times the fraction } W/Z$$

where W is the number of nucleotides scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of C and D, and where Z is the total number of nucleotides in D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C. As examples of % nucleic acid sequence identity calculations, Tables 4 and 5, demonstrate how to calculate the % nucleic acid sequence identity of the nucleic acid sequence designated "Comparison DNA" to the nucleic acid sequence designated "PRO-DNA", wherein "PRO-DNA" represents a hypothetical PRO-encoding nucleic acid sequence of interest, "Comparison DNA" represents the nucleotide sequence of a nucleic acid molecule against which the "PRO-DNA" nucleic acid molecule of interest is being compared, and "N", "L" and "V" each represent different hypothetical nucleotides.

Unless specifically stated otherwise, all % nucleic acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program. However, % nucleic acid sequence identity values may also be obtained as described below by using the WU-BLAST-2 computer program (Altschul et al., Methods in Enzymology 266:460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, i.e., the adjustable parameters, are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11, and scoring matrix = BLOSUM62. When WU-BLAST-2 is employed, a % nucleic acid sequence identity value is determined by dividing (a) the number of matching identical nucleotides between the nucleic acid sequence of the PRO polypeptide-encoding nucleic acid molecule of interest having a sequence derived from the native sequence PRO polypeptide-encoding nucleic acid and the comparison nucleic acid molecule of interest (i.e., the sequence against which the PRO polypeptide-encoding nucleic acid molecule of interest is being compared which may be a variant PRO polynucleotide) as determined by WU-BLAST-2 by (b) the total number of nucleotides of the PRO polypeptide-encoding nucleic acid molecule of interest. For example, in the statement "an isolated nucleic acid molecule comprising a nucleic acid sequence A which has or having at least 80% nucleic acid sequence identity to the nucleic acid sequence B", the nucleic acid sequence A is the comparison nucleic acid molecule of interest and the nucleic acid sequence B is the nucleic acid sequence of the PRO polypeptide-encoding nucleic acid molecule of interest.



Percent nucleic acid sequence identity may also be determined using the sequence comparison program NCBI-BLAST2 (Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997)). The NCBI-BLAST2 sequence comparison program may be downloaded from <http://www.ncbi.nlm.nih.gov> or otherwise obtained from the National Institute of Health, Bethesda, MD. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

In situations where NCBI-BLAST2 is employed for sequence comparisons, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

$$100 \text{ times the fraction } W/Z$$

where W is the number of nucleotides scored as identical matches by the sequence alignment program NCBI-BLAST2 in that program's alignment of C and D, and where Z is the total number of nucleotides in D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C.

In other embodiments, PRO variant polynucleotides are nucleic acid molecules that encode an active PRO polypeptide and which are capable of hybridizing, preferably under stringent hybridization and wash conditions, to nucleotide sequences encoding a full-length PRO polypeptide as disclosed herein. PRO variant polypeptides may be those that are encoded by a PRO variant polynucleotide.

The term "positives", in the context of sequence comparison performed as described above, includes residues in the sequences compared that are not identical but have similar properties (e.g. as a result of conservative substitutions, see Table 6 below). For purposes herein, the % value of positives is determined by dividing (a) the number of amino acid residues scoring a positive value between the PRO polypeptide amino acid sequence of interest having a sequence derived from the native PRO polypeptide sequence and the comparison amino acid sequence of interest (i.e., the amino acid sequence against which the PRO polypeptide sequence is being compared) as determined in the BLOSUM62 matrix of WU-BLAST-2 by (b) the total number of amino acid residues of the PRO polypeptide of interest.

Unless specifically stated otherwise, the % value of positives is calculated as described in the immediately preceding paragraph. However, in the context of the amino acid sequence identity comparisons performed as described for ALIGN-2 and NCBI-BLAST-2 above, includes amino acid residues in the sequences compared that are not only identical, but also those that have similar properties. Amino acid residues that score a positive value to an amino acid residue of interest are those that are either identical to the amino acid residue of interest or are a preferred substitution (as defined in Table 6 below) of the amino acid residue of interest.

For amino acid sequence comparisons using ALIGN-2 or NCBI-BLAST2, the % value of positives of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % positives to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scoring a positive value as defined above by the sequence alignment program ALIGN-2 or NCBI-BLAST2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % positives of A to B will not equal the % positives of B to A.

"Isolated," when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the PRO polypeptide natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "isolated" PRO polypeptide-encoding nucleic acid or other polypeptide-encoding nucleic acid is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the polypeptide-encoding nucleic acid. An isolated polypeptide-encoding nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated polypeptide-encoding nucleic acid molecules therefore are distinguished from the specific polypeptide-encoding nucleic acid molecule as it exists in natural cells. However, an isolated polypeptide-encoding nucleic acid molecule includes polypeptide-encoding nucleic acid molecules contained in cells that ordinarily express the polypeptide where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter

or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

The term "antibody" is used in the broadest sense and specifically covers, for example, single anti-PRO monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies), anti-PRO antibody compositions with polyepitopic specificity, single chain anti-PRO antibodies, and fragments of anti-PRO antibodies (see below). The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995).

"Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1 % sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

"Moderately stringent conditions" may be identified as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and %SDS) less stringent than those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA,

followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a PRO polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (i.e., is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

"Active" or "activity" for the purposes herein refers to form(s) of a PRO polypeptide which retain a biological and/or an immunological activity of native or naturally-occurring PRO, wherein "biological" activity refers to a biological function (either inhibitory or stimulatory) caused by a native or naturally-occurring PRO other than the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring PRO and an "immunological" activity refers to the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring PRO.

The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native PRO polypeptide disclosed herein. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native PRO polypeptide disclosed herein. Suitable agonist or antagonist molecules specifically include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native PRO polypeptides, peptides, antisense oligonucleotides, small organic molecules, etc. Methods for identifying agonists or antagonists of a PRO polypeptide may comprise contacting a PRO polypeptide with a candidate agonist or antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the PRO polypeptide.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats, rabbits, etc. Preferably, the mammal is human.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')<sub>2</sub>, and Fv fragments; diabodies; linear antibodies (Zapata et al., Protein Eng. 8(10): 1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an F(ab')<sub>2</sub> fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V<sub>H</sub>-V<sub>L</sub> dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains

bear a free thiol group. F(ab')<sub>2</sub> antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains.

5 Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2.

10 "Single-chain Fv" or "sFv" antibody fragments comprise the V<sub>H</sub> and V<sub>L</sub> domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V<sub>H</sub> and V<sub>L</sub> domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

15 The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V<sub>H</sub>) connected to a light-chain variable domain (V<sub>L</sub>) in the same polypeptide chain (V<sub>H</sub>-V<sub>L</sub>). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993).

20 An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

30 The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

35 By "solid phase" is meant a non-aqueous matrix to which the antibody of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (e.g., controlled pore glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay

plate; in others it is a purification column (e.g., an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as a PRO polypeptide or antibody thereto) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

A "small molecule" is defined herein to have a molecular weight below about 500 Daltons.

"FGFR-1", "FGFR-2", "FGFR-3" and FGFR-4" refer to the fibroblast growth factor receptors 1, 2, 3 and 4, respectively, as disclosed by Isacchi et al., Nuc. Acids Res. 18(7):1906 (1990), Dionne et al., EMBO J. 9(9):2685-2692 (1990), Keegan et al., Proc. Natl. Acad. Sci. USA 88:1095-1099 (1991) and Partanen et al., EMBO J. 10(6):1347-1354 (1991), respectively.

**Table 1**

```

/*
*
* C-C increased from 12 to 15
* Z is average of EQ
5  * B is average of ND
* match with stop is _M; stop-stop = 0; J (joker) match = 0
*/
#define _M      -8      /* value of a match with a stop */

10 int      _day[26][26] = {
/*      A B C D E F G H I J K L M N O P Q R S T U V W X Y Z */
/* A */      { 2, 0,-2, 0, 0,-4, 1,-1,-1, 0,-1,-2,-1, 0,_M, 1, 0,-2, 1, 1, 0, 0,-6, 0,-3, 0},
/* B */      { 0, 3,-4, 3, 2,-5, 0, 1,-2, 0, 0,-3,-2, 2,_M,-1, 1, 0, 0, 0, 0,-2,-5, 0,-3, 1},
/* C */      {-2,-4,15,-5,-5,-4,-3,-3,-2, 0,-5,-6,-5,-4,_M,-3,-5,-4, 0,-2, 0,-2,-8, 0, 0,-5},
15 /* D */      { 0, 3,-5, 4, 3,-6, 1, 1,-2, 0, 0,-4,-3, 2,_M,-1, 2,-1, 0, 0, 0,-2,-7, 0,-4, 2},
/* E */      { 0, 2,-5, 3, 4,-5, 0, 1,-2, 0, 0,-3,-2, 1,_M,-1, 2,-1, 0, 0, 0,-2,-7, 0,-4, 3},
/* F */      {-4,-5,-4,-6,-5, 9,-5,-2, 1, 0,-5, 2, 0,-4,_M,-5,-5,-4,-3,-3, 0,-1, 0, 0, 7,-5},
/* G */      { 1, 0,-3, 1, 0,-5, 5,-2,-3, 0,-2,-4,-3, 0,_M,-1,-1,-3, 1, 0, 0,-1,-7, 0,-5, 0},
/* H */      {-1, 1,-3, 1, 1,-2,-2, 6,-2, 0, 0,-2,-2, 2,_M, 0, 3, 2,-1,-1, 0,-2,-3, 0, 0, 2},
20 /* I */      {-1,-2,-2,-2,-2, 1,-3,-2, 5, 0,-2, 2, 2,-2,_M,-2,-2,-2,-1, 0, 0, 4,-5, 0,-1,-2},
/* J */      { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,_M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* K */      {-1, 0,-5, 0, 0,-5,-2, 0,-2, 0, 5,-3, 0, 1,_M,-1, 1, 3, 0, 0, 0,-2,-3, 0,-4, 0},
/* L */      {-2,-3,-6,-4,-3, 2,-4,-2, 2, 0,-3, 6, 4,-3,_M,-3,-2,-3,-3,-1, 0, 2,-2, 0,-1,-2},
/* M */      {-1,-2,-5,-3,-2, 0,-3,-2, 2, 0, 0, 4, 6,-2,_M,-2,-1, 0,-2,-1, 0, 2,-4, 0,-2,-1},
25 /* N */      { 0, 2,-4, 2, 1,-4, 0, 2,-2, 0, 1,-3,-2, 2,_M,-1, 1, 0, 1, 0, 0,-2,-4, 0,-2, 1},
/* O */      {_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M, 0,_M,_M,_M,_M,_M,_M,_M,_M,_M},
/* P */      { 1,-1,-3,-1,-1,-5,-1, 0,-2, 0,-1,-3,-2,-1,_M, 6, 0, 0, 1, 0, 0,-1,-6, 0,-5, 0},
/* Q */      { 0, 1,-5, 2, 2,-5,-1, 3,-2, 0, 1,-2,-1, 1,_M, 0, 4, 1,-1,-1, 0,-2,-5, 0,-4, 3},
/* R */      {-2, 0,-4,-1,-1,-4,-3, 2,-2, 0, 3,-3, 0, 0,_M, 0, 1, 6, 0,-1, 0,-2, 2, 0,-4, 0},
30 /* S */      { 1, 0, 0, 0, 0,-3, 1,-1,-1, 0, 0,-3,-2, 1,_M, 1,-1, 0, 2, 1, 0,-1,-2, 0,-3, 0},
/* T */      { 1, 0,-2, 0, 0,-3, 0,-1, 0, 0, 0,-1,-1, 0,_M, 0,-1,-1, 1, 3, 0, 0,-5, 0,-3, 0},
/* U */      { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,_M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* V */      { 0,-2,-2,-2,-2,-1,-1,-2, 4, 0,-2, 2, 2,-2,_M,-1,-2,-2,-1, 0, 0, 4,-6, 0,-2,-2},
/* W */      {-6,-5,-8,-7,-7, 0,-7,-3,-5, 0,-3,-2,-4,-4,_M,-6,-5, 2,-2,-5, 0,-6,17, 0, 0,-6},
35 /* X */      { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,_M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* Y */      {-3,-3, 0,-4,-4, 7,-5, 0,-1, 0,-4,-1,-2,-2,_M,-5,-4,-4,-3,-3, 0,-2, 0, 0,10,-4},
/* Z */      { 0, 1,-5, 2, 3,-5, 0, 2,-2, 0, 0,-2,-1, 1,_M, 0, 3, 0, 0, 0, 0,-2,-6, 0,-4, 4}
};

40

45

50

55

```



Table 1 (cont')

```

/*
*/
#include <stdio.h>
#include <ctype.h>

#define MAXJMP      16      /* max jumps in a diag */
#define MAXGAP      24      /* don't continue to penalize gaps larger than this */
#define JMPS        1024    /* max jmps in an path */
#define MX          4       /* save if there's at least MX-1 bases since last jmp */

#define DMAT         3      /* value of matching bases */
#define DMIS         0      /* penalty for mismatched bases */
#define DINS0        8      /* penalty for a gap */
#define DINS1        1      /* penalty per base */
#define PINS0        8      /* penalty for a gap */
#define PINS1        4      /* penalty per residue */

struct jmp {
    short          n[MAXJMP]; /* size of jmp (neg for dely) */
    unsigned short x[MAXJMP]; /* base no. of jmp in seq x */
}; /* limits seq to 2^16 - 1 */

struct diag {
    int            score;      /* score at last jmp */
    long           offset;     /* offset of prev block */
    short          ijmp;       /* current jmp index */
    struct jmp      jp;        /* list of jmps */
};

struct path {
    int            spc;        /* number of leading spaces */
    short          n[JMPS];    /* size of jmp (gap) */
    int            x[JMPS];    /* loc of jmp (last elem before gap) */
};

char             *ofile;      /* output file name */
char             *namex[2];   /* seq names: getseqs() */
char             *prog;       /* prog name for err msgs */
char             *seqx[2];    /* seqs: getseqs() */
int              dmax;        /* best diag: nw() */
int              dmax0;       /* final diag */
int              dna;         /* set if dna: main() */
int              endgaps;     /* set if penalizing end gaps */
int              gapx, gapy;   /* total gaps in seqs */
int              len0, len1;   /* seq lens */
int              ngapx, ngapy; /* total size of gaps */
int              smax;        /* max score: nw() */
int              *xbm;        /* bitmap for matching */
long             offset;      /* current offset in jmp file */
struct diag      *dx;         /* holds diagonals */
struct path      pp[2];       /* holds path for seqs */

char             *calloc(), *malloc(), *index(), *strcpy();
char             *getseq(), *g_calloc();

```

## Table 1 (cont')

```

/* Needleman-Wunsch alignment program
*
* usage: progs file1 file2
* where file1 and file2 are two dna or two protein sequences.
* The sequences can be in upper- or lower-case and may contain ambiguity
* Any lines beginning with ';', '>' or '<' are ignored
* Max file length is 65535 (limited by unsigned short x in the jmp struct)
* A sequence with 1/3 or more of its elements ACGTU is assumed to be DNA
* Output is in the file "align.out"
*
* The program may create a tmp file in /tmp to hold info about traceback.
* Original version developed under BSD 4.3 on a vax 8650
*/
#include "nw.h"
#include "day.h"

static _dbval[26] = {
    1, 14, 2, 13, 0, 0, 4, 11, 0, 0, 12, 0, 3, 15, 0, 0, 0, 5, 6, 8, 8, 7, 9, 0, 10, 0
};

static _pbval[26] = {
    1, 2 | (1 < < ('D'-'A')) | (1 < < ('N'-'A')), 4, 8, 16, 32, 64,
    128, 256, 0xFFFFFFFF, 1 < < 10, 1 < < 11, 1 < < 12, 1 < < 13, 1 < < 14,
    1 < < 15, 1 < < 16, 1 < < 17, 1 < < 18, 1 < < 19, 1 < < 20, 1 < < 21, 1 < < 22,
    1 < < 23, 1 < < 24, 1 < < 25 | (1 < < ('E'-'A')) | (1 < < ('Q'-'A'))
};

main(ac, av)
    int ac;
    char *av[];
{
    prog = av[0];
    if (ac != 3) {
        fprintf(stderr, "usage: %s file1 file2\n", prog);
        fprintf(stderr, "where file1 and file2 are two dna or two protein sequences.\n");
        fprintf(stderr, "The sequences can be in upper- or lower-case\n");
        fprintf(stderr, "Any lines beginning with ';', '>' or '<' are ignored\n");
        fprintf(stderr, "Output is in the file \"align.out\"\n");
        exit(1);
    }
    namex[0] = av[1];
    namex[1] = av[2];
    seqx[0] = getseq(namex[0], &len0);
    seqx[1] = getseq(namex[1], &len1);
    xbm = (dna)? _dbval : _pbval;

    endgaps = 0; /* 1 to penalize endgaps */
    ofile = "align.out"; /* output file */

    nw(); /* fill in the matrix, get the possible jumps */
    readjumps(); /* get the actual jumps */
    print(); /* print stats, alignment */

    cleanup(0); /* unlink any tmp files */
}

```

**Table 1 (cont')**

```

/* do the alignment, return best score: main()
* dna: values in Fitch and Smith, PNAS, 80, 1382-1386, 1983
* pro: PAM 250 values
* When scores are equal, we prefer mismatches to any gap, prefer
5  * a new gap to extending an ongoing gap, and prefer a gap in seqx
  * to a gap in seq y.
  */
nw()
{
10  char      *px, *py;          /* seqs and ptrs */
    int      *ndely, *dely;     /* keep track of dely */
    int      ndelx, delx;       /* keep track of delx */
    int      *tmp;              /* for swapping row0, row1 */
    int      mis;               /* score for each type */
15  int      ins0, ins1;         /* insertion penalties */
    register id;                /* diagonal index */
    register ij;                /* jmp index */
    register *col0, *col1;      /* score for curr, last row */
    register xx, yy;            /* index into seqs */

20  dx = (struct diag *)g_calloc("to get diags", len0+len1+1, sizeof(struct diag));

    ndely = (int *)g_calloc("to get ndely", len1+1, sizeof(int));
    dely = (int *)g_calloc("to get dely", len1+1, sizeof(int));
25  col0 = (int *)g_calloc("to get col0", len1+1, sizeof(int));
    col1 = (int *)g_calloc("to get col1", len1+1, sizeof(int));
    ins0 = (dna)? DINS0 : PINS0;
    ins1 = (dna)? DINS1 : PINS1;

30  smax = -10000;
    if (endgaps) {
        for (col0[0] = dely[0] = -ins0, yy = 1; yy <= len1; yy++) {
            col0[yy] = dely[yy] = col0[yy-1] - ins1;
            ndely[yy] = yy;
35  }
        col0[0] = 0;          /* Waterman Bull Math Biol 84 */
    }
    else
40  for (yy = 1; yy <= len1; yy++)
        dely[yy] = -ins0;

    /* fill in match matrix
    */
45  for (px = seqx[0], xx = 1; xx <= len0; px++, xx++) {
        /* initialize first entry in col
        */
        if (endgaps) {
            if (xx == 1)
50  col1[0] = delx = -(ins0+ins1);
            else
                col1[0] = delx = col0[0] - ins1;
            ndelx = xx;
        }
        else {
55  col1[0] = 0;
            delx = -ins0;
            ndelx = 0;
        }
60

```

**nw**

**Table 1 (cont')**

...nw

```

5  for (py = seqx[1], yy = 1; yy <= len1; py++, yy++) {
    mis = col0[yy-1];
    if (dna)
        mis += (xbm[*px-'A']&xbm[*py-'A'])? DMAT : DMIS;
    else
        mis += _day[*px-'A'][*py-'A'];

10  /* update penalty for del in x seq;
    * favor new del over ongoing del
    * ignore MAXGAP if weighting endgaps
    */
    if (endgaps || ndely[yy] < MAXGAP) {
15         if (col0[yy] - ins0 >= dely[yy]) {
            dely[yy] = col0[yy] - (ins0+ins1);
            ndely[yy] = 1;
        } else {
            dely[yy] -= ins1;
            ndely[yy]++;
20         }
    } else {
        if (col0[yy] - (ins0+ins1) >= dely[yy]) {
            dely[yy] = col0[yy] - (ins0+ins1);
            ndely[yy] = 1;
25         } else
            ndely[yy]++;
    }

    /* update penalty for del in y seq;
    * favor new del over ongoing del
    */
    if (endgaps || ndelx < MAXGAP) {
30         if (col1[yy-1] - ins0 >= delx) {
            delx = col1[yy-1] - (ins0+ins1);
            ndelx = 1;
35         } else {
            delx -= ins1;
            ndelx++;
        }
    } else {
40         if (col1[yy-1] - (ins0+ins1) >= delx) {
            delx = col1[yy-1] - (ins0+ins1);
            ndelx = 1;
        } else
45         ndelx++;
    }

    /* pick the maximum score; we're favoring
    * mis over any del and delx over dely
    */
50
55
60

```

**Table 1 (cont')**

...nw

```

id = xx - yy + len1 - 1;
if (mis >= delx && mis >= dely[yy])
    col1[yy] = mis;
5   else if (delx >= dely[yy]) {
        col1[yy] = delx;
        ij = dx[id].ijmp;
        if (dx[id].jp.n[0] && (!dna || (ndelx >= MAXJMP
10      && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
            dx[id].ijmp++;
            if (++ij >= MAXJMP) {
                writejumps(id);
                ij = dx[id].ijmp = 0;
                dx[id].offset = offset;
                offset += sizeof(struct jmp) + sizeof(offset);
            }
            dx[id].jp.n[ij] = ndelx;
            dx[id].jp.x[ij] = xx;
            dx[id].score = delx;
        }
        else {
            col1[yy] = dely[yy];
            ij = dx[id].ijmp;
25      if (dx[id].jp.n[0] && (!dna || (ndely[yy] >= MAXJMP
            && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
                dx[id].ijmp++;
                if (++ij >= MAXJMP) {
                    writejumps(id);
                    ij = dx[id].ijmp = 0;
                    dx[id].offset = offset;
                    offset += sizeof(struct jmp) + sizeof(offset);
                }
            }
            dx[id].jp.n[ij] = -ndely[yy];
            dx[id].jp.x[ij] = xx;
            dx[id].score = dely[yy];
        }
        if (xx == len0 && yy < len1) {
40          /* last col
            */
            if (endgaps)
                col1[yy] -= ins0+ins1*(len1-yy);
            if (col1[yy] > smax) {
45              smax = col1[yy];
              dmax = id;
            }
        }
    }
50   if (endgaps && xx < len0)
        col1[yy-1] -= ins0+ins1*(len0-xx);
    if (col1[yy-1] > smax) {
        smax = col1[yy-1];
        dmax = id;
55   }
    tmp = col0; col0 = col1; col1 = tmp;
}
(void) free((char *)ndely);
(void) free((char *)dely);
(void) free((char *)col0);
60  (void) free((char *)col1);
    }
```

**Table 1 (cont')**

```

/*
 *
 * print() -- only routine visible outside this module
 *
5  * static:
 * getmat() -- trace back best path, count matches: print()
 * pr_align() -- print alignment of described in array p[]: print()
 * dumpblock() -- dump a block of lines with numbers, stars: pr_align()
 * nums() -- put out a number line: dumpblock()
10 * putline() -- put out a line (name, {num}, seq, {num}): dumpblock()
 * stars() -- put a line of stars: dumpblock()
 * stripname() -- strip any path and prefix from a seqname
 */

15 #include "nw.h"

#define SPC      3
#define P_LINE  256 /* maximum output line */
#define P_SPC    3   /* space between name or num and seq */

20 extern _day[26][26];
int olen; /* set output line length */
FILE *fx; /* output file */

25 print()
{
    int lx, ly, firstgap, lastgap; /* overlap */

    if ((fx = fopen(ofile, "w")) == 0) {
30         fprintf(stderr, "%s: can't write %s\n", prog, ofile);
        cleanup(1);
    }
    fprintf(fx, "< first sequence: %s (length = %d)\n", namex[0], len0);
    fprintf(fx, "< second sequence: %s (length = %d)\n", namex[1], len1);
35     olen = 60;
    lx = len0;
    ly = len1;
    firstgap = lastgap = 0;
    if (dmax < len1 - 1) { /* leading gap in x */
40         pp[0].spc = firstgap = len1 - dmax - 1;
        ly -= pp[0].spc;
    }
    else if (dmax > len1 - 1) { /* leading gap in y */
        pp[1].spc = firstgap = dmax - (len1 - 1);
45         lx -= pp[1].spc;
    }
    if (dmax0 < len0 - 1) { /* trailing gap in x */
        lastgap = len0 - dmax0 - 1;
        lx -= lastgap;
50     }
    else if (dmax0 > len0 - 1) { /* trailing gap in y */
        lastgap = dmax0 - (len0 - 1);
        ly -= lastgap;
    }
55     getmat(lx, ly, firstgap, lastgap);
    pr_align();
}

60

```

print

**Table 1 (cont')**

```

/*
 * trace back the best path, count matches
 */
static
5 getmat(lx, ly, firstgap, lastgap)                                getmat
    int      lx, ly;                                /* "core" (minus endgaps) */
    int      firstgap, lastgap;                      /* leading trailing overlap */
{
    int      nm, i0, i1, siz0, siz1;
10    char     outx[32];
    double   pct;
    register n0, n1;
    register char *p0, *p1;

15    /* get total matches, score
    */
    i0 = i1 = siz0 = siz1 = 0;
    p0 = seqx[0] + pp[1].spc;
    p1 = seqx[1] + pp[0].spc;
20    n0 = pp[1].spc + 1;
    n1 = pp[0].spc + 1;

    nm = 0;
    while ( *p0 && *p1 ) {
25         if (siz0) {
             p1++;
             n1++;
             siz0--;
         }
30         else if (siz1) {
             p0++;
             n0++;
             siz1--;
         }
35         else {
             if (xbm[*p0-'A']&xbm[*p1-'A'])
                 nm++;
             if (n0++ == pp[0].x[i0])
40                 siz0 = pp[0].n[i0++];
             if (n1++ == pp[1].x[i1])
                 siz1 = pp[1].n[i1++];
             p0++;
             p1++;
         }
45     }

    /* pct homology:
    * if penalizing endgaps, base is the shorter seq
    * else, knock off overhangs and take shorter core
    */
50    if (endgaps)
        lx = (len0 < len1)? len0 : len1;
    else
        lx = (lx < ly)? lx : ly;
55    pct = 100.*((double)nm)/((double)lx);
    fprintf(fx, "\n");
    fprintf(fx, "< %d match%s in an overlap of %d: %.2f percent similarity\n",
        nm, (nm == 1)? "" : "es", lx, pct);
60

```

**Table 1 (cont')**

...getmat

```

fprintf(fx, "< gaps in first sequence: %d", gapx);
if (gapx) {
    (void) sprintf(outx, " (%d %s%s)",
        ngapx, (dna)? "base":"residue", (ngapx == 1)? "" : "s");
    fprintf(fx, "%s", outx);

    fprintf(fx, ", gaps in second sequence: %d", gapy);
    if (gapy) {
        (void) sprintf(outx, " (%d %s%s)",
            ngapy, (dna)? "base":"residue", (ngapy == 1)? "" : "s");
        fprintf(fx, "%s", outx);
    }
    if (dna)
        fprintf(fx,
            "\n< score: %d (match = %d, mismatch = %d, gap penalty = %d + %d per base)\n",
            smax, DMAT, DMIS, DINS0, DINS1);
    else
        fprintf(fx,
            "\n< score: %d (Dayhoff PAM 250 matrix, gap penalty = %d + %d per residue)\n",
            smax, PINS0, PINS1);
    if (endgaps)
        fprintf(fx,
            "< endgaps penalized. left endgap: %d %s%s, right endgap: %d %s%s\n",
            firstgap, (dna)? "base" : "residue", (firstgap == 1)? "" : "s",
            lastgap, (dna)? "base" : "residue", (lastgap == 1)? "" : "s");
    else
        fprintf(fx, "< endgaps not penalized\n");
}

static      nm;          /* matches in core -- for checking */
static      lmax;        /* lengths of stripped file names */
static      ij[2];       /* jmp index for a path */
static      nc[2];       /* number at start of current line */
static      ni[2];       /* current elem number -- for gapping */
static      siz[2];
static char *ps[2];      /* ptr to current element */
static char *po[2];      /* ptr to next output char slot */
static char out[2][P_LINE]; /* output line */
static char star[P_LINE]; /* set by stars() */

/*
 * print alignment of described in struct path pp[]
 */
static
pr_align()
{
    int      nn;          /* char count */
    int      more;
    register i;

    for (i = 0, lmax = 0; i < 2; i++) {
        nn = stripname(name[i]);
        if (nn > lmax)
            lmax = nn;

        nc[i] = 1;
        ni[i] = 1;
        siz[i] = ij[i] = 0;
        ps[i] = seqx[i];
        po[i] = out[i];
    }

```

pr\_align



**Table 1 (cont')**

**...pr\_align**

```

5      for (nn = nm = 0, more = 1; more; ) {
        for (i = more = 0; i < 2; i++) {
            /*
            * do we have more of this sequence?
            */
            if (!*ps[i])
                continue;

10         more++;

            if (pp[i].spc) { /* leading space */
                *po[i]++ = ' ';
                pp[i].spc--;
15         }
            else if (siz[i]) { /* in a gap */
                *po[i]++ = '-';
                siz[i]--;
20         }
            else { /* we're putting a seq element
                */
                *po[i] = *ps[i];
                if (islower(*ps[i]))
                    *ps[i] = toupper(*ps[i]);
25         po[i]++;
                ps[i]++;

                /*
                * are we at next gap for this seq?
                */
                if (ni[i] == pp[i].x[ij[i]]) {
                    /*
                    * we need to merge all gaps
                    * at this location
                    */
35         siz[i] = pp[i].n[ij[i] + +];
                    while (ni[i] == pp[i].x[ij[i]])
                        siz[i] += pp[i].n[ij[i] + +];
40         }
                    ni[i]++;
                }
            }
        }
        if (++nn == olen || !more && nn) {
            dumpblock();
            for (i = 0; i < 2; i++)
                po[i] = out[i];
            nn = 0;
        }
50     }

    /*
    * dump a block of lines, including numbers, stars: pr_align()
    */
55     static
    dumpblock()
    {
        register i;

60         for (i = 0; i < 2; i++)
            *po[i]-- = '\0';

```

**dumpblock**

## Table 1 (cont')

...dumpblock

```

5      (void) putc('\n', fx);
      for (i = 0; i < 2; i++) {
          if (*out[i] && (*out[i] != ' ' || *(po[i]) != ' ')) {
              if (i == 0)
                  nums(i);
              if (i == 0 && *out[1])
                  stars();
10         putline(i);
              if (i == 0 && *out[1])
                  fprintf(fx, star);
              if (i == 1)
                  nums(i);
15         }
      }
  }

```

```

20  /*
    * put out a number line: dumpblock()
    */

```

static

nums(ix)

nums

```

25  {
      int      ix;      /* index in out[] holding seq line */
      char      nline[P_LINE];
      register  i, j;
      register char *pn, *px, *py;

30      for (pn = nline, i = 0; i < lmax+P_SPC; i++, pn++)
          *pn = ' ';
      for (i = nc[ix], py = out[ix]; *py; py++, pn++) {
          if (*py == ' ' || *py == '-')
              *pn = ' ';
35          else {
              if (i%10 == 0 || (i == 1 && nc[ix] != 1)) {
                  j = (i < 0)? -i : i;
                  for (px = pn; j; j /= 10, px--)
                      *px = j%10 + '0';
40                  if (i < 0)
                      *px = '-';

                  }
              else
                  *pn = ' ';
45          i++;
          }
      }
      *pn = '\0';
      nc[ix] = i;
50      for (pn = nline; *pn; pn++)
          (void) putc(*pn, fx);
      (void) putc('\n', fx);
  }

```

```

55  /*
    * put out a line (name, [num], seq, [num]): dumpblock()
    */

```

static

putline(ix)

putline

```

60  {
      int      ix;

```

**Table 1 (cont')**

**...putline**

```

5      int          i;
      register char *px;

      for (px = namex[ix], i = 0; *px && *px != ':'; px++, i++)
          (void) putc(*px, fx);
      for (; i < lmax+P_SPC; i++)
          (void) putc(' ', fx);

10     /* these count from 1:
       * ni[] is current element (from 1)
       * nc[] is number at start of current line
       */

15     for (px = out[ix]; *px; px++)
          (void) putc(*px&0x7F, fx);
      (void) putc('\n', fx);
  }

20  /*
   * put a line of stars (seqs always in out[0], out[1]): dumpblock()
   */
  static
25  stars()
  {
      int          i;
      register char *p0, *p1, cx, *px;

30     if (!*out[0] || (*out[0] == ' ' && *(p0[0]) == ' ') ||
        !*out[1] || (*out[1] == ' ' && *(p0[1]) == ' '))
          return;
      px = star;
      for (i = lmax+P_SPC; i; i--)
35         *px++ = ' ';

      for (p0 = out[0], p1 = out[1]; *p0 && *p1; p0++, p1++) {
          if (isalpha(*p0) && isalpha(*p1)) {

40                 if (xbm[*p0-'A']&xbm[*p1-'A']) {
                        cx = '*';
                        nm++;
                    }
                    else if (!dna && _day[*p0-'A'][*p1-'A'] > 0)
45                         cx = '.';
                    else
                        cx = ' ';
                }
                else
50                     cx = ' ';
                *px++ = cx;
            }
            *px++ = '\n';
            *px = '\0';
55     }

```

**stars**

**Table 1 (cont')**

	/*	
	* strip path or prefix from pn, return len: pr_align()	
	*/	
	static	
5	stripname(pn)	stripname
	char *pn; /* file name (may be path) */	
	{	
	register char *px, *py;	
10	py = 0;	
	for (px = pn; *px; px++)	
	if (*px == '/')	
	py = px + 1;	
15	if (py)	
	(void) strcpy(pn, py);	
	return(strlen(pn));	
	}	
20		
25		
30		
35		
40		
45		
50		
55		
60		

**Table 1 (cont')**

```

/*
 * cleanup() -- cleanup any tmp file
 * getseq() -- read in seq, set dna, len, maxlen
 * g_calloc() -- calloc() with error checkin
5  * readjumps() -- get the good jumps, from tmp file if necessary
 * writejumps() -- write a filled array of jumps to a tmp file: nw()
 */
#include "nw.h"
#include <sys/file.h>

10 char    *jname = "/tmp/homgXXXXXX";          /* tmp file for jumps */
FILE      *fj;

int       cleanup();                          /* cleanup tmp file */
15 long    lseek();

/*
 * remove any tmp file if we blow
 */
20 cleanup(i)                                cleanup
{
    int      i;
    if (fj)
        (void) unlink(jname);
25     exit(i);
}

/*
 * read, return ptr to seq, set dna, len, maxlen
 * skip lines starting with ';', '<', or '>'
 * seq in upper or lower case
 */
30 char    *
getseq(file, len)                                getseq
35     char    *file;      /* file name */
    int      *len;        /* seq len */
{
    char      line[1024], *pseq;
    register char *px, *py;
    int       natgc, tlen;
    FILE      *fp;

    if ((fp = fopen(file, "r")) == 0) {
45         fprintf(stderr, "%s: can't read %s\n", prog, file);
        exit(1);
    }
    tlen = natgc = 0;
    while (fgets(line, 1024, fp)) {
50         if (*line == ';' || *line == '<' || *line == '>')
            continue;
        for (px = line; *px != '\n'; px++)
            if (isupper(*px) || islower(*px))
                tlen++;
    }
55     if ((pseq = malloc((unsigned)(tlen+6))) == 0) {
        fprintf(stderr, "%s: malloc() failed to get %d bytes for %s\n", prog, tlen+6, file);
        exit(1);
    }
    pseq[0] = pseq[1] = pseq[2] = pseq[3] = '\0';
60

```

**Table 1 (cont')**

...getseq

```

py = pseq + 4;
*len = tlen;
rewind(fp);

5
while (fgets(line, 1024, fp)) {
    if (*line == ';' || *line == '<' || *line == '>')
        continue;
    for (px = line; *px != '\n'; px++) {
10
        if (isupper(*px))
            *py++ = *px;
        else if (islower(*px))
            *py++ = toupper(*px);
        if (index("ATGCU",*(py-1)))
            natgc++;
15
    }
    *py++ = '\0';
    *py = '\0';
    (void) fclose(fp);
    dna = natgc > (tlen/3);
    return(pseq+4);
20
}

25
char *
g_alloc(msg, nx, sz)
char *msg; /* program, calling routine */
int nx, sz; /* number and size of elements */

30
{
    char *px, *calloc();

    if ((px = calloc((unsigned)nx, (unsigned)sz)) == 0) {
        if (*msg) {
35
            fprintf(stderr, "%s: g_alloc() failed %s (n=%d, sz=%d)\n", prog, msg, nx, sz);
            exit(1);
        }
    }
    return(px);
40
}

/*
 * get final jmps from dx[] or tmp file, set pp[], reset dmax: main()
 */

45
readjmps()
{
    int fd = -1;
    int siz, i0, i1;
    register i, j, xx;

50
    if (!f) {
        (void) fclose(f);
        if ((fd = open(jname, O_RDONLY, 0)) < 0) {
            fprintf(stderr, "%s: can't open() %s\n", prog, jname);
            cleanup(1);
55
        }
    }
    for (i = i0 = i1 = 0, dmax0 = dmax, xx = len0; ; i++) {
        while (1) {
60
            for (j = dx[dmax].ijmp; j >= 0 && dx[dmax].jp.x[j] >= xx; j--);

```

**g\_alloc**

**readjmps**

...readjumps

```

5      if (j < 0 && dx[dmax].offset && fj) {
        (void) lseek(fd, dx[dmax].offset, 0);
        (void) read(fd, (char *)&dx[dmax].jp, sizeof(struct jmp));
        (void) read(fd, (char *)&dx[dmax].offset, sizeof(dx[dmax].offset));
        dx[dmax].ijmp = MAXJMP-1;
      }
      else
10         break;
    }
    if (i >= JMPS) {
        fprintf(stderr, "%s: too many gaps in alignment\n", prog);
        cleanup(1);
    }
15    if (j >= 0) {
        siz = dx[dmax].jp.n[j];
        xx = dx[dmax].jp.x[j];
        dmax += siz;
        if (siz < 0) { /* gap in second seq */
20            pp[1].n[i1] = -siz;
            xx += siz;
            /* id = xx - yy + len1 - 1
             */
            pp[1].x[i1] = xx - dmax + len1 - 1;
            gapy++;
            ngapy -= siz;
        /* ignore MAXGAP when doing endgaps */
        siz = (-siz < MAXGAP || endgaps)? -siz : MAXGAP;
        i1++;
30        }
        else if (siz > 0) { /* gap in first seq */
            pp[0].n[i0] = siz;
            pp[0].x[i0] = xx;
            gapx++;
            ngapx += siz;
35        /* ignore MAXGAP when doing endgaps */
            siz = (siz < MAXGAP || endgaps)? siz : MAXGAP;
            i0++;
        }
40        }
        else
            break;
    }

45    /* reverse the order of jumps
    */
    for (j = 0, i0--; j < i0; j++, i0--) {
        i = pp[0].n[j]; pp[0].n[j] = pp[0].n[i0]; pp[0].n[i0] = i;
        i = pp[0].x[j]; pp[0].x[j] = pp[0].x[i0]; pp[0].x[i0] = i;
50    }
    for (j = 0, i1--; j < i1; j++, i1--) {
        i = pp[1].n[j]; pp[1].n[j] = pp[1].n[i1]; pp[1].n[i1] = i;
        i = pp[1].x[j]; pp[1].x[j] = pp[1].x[i1]; pp[1].x[i1] = i;
55    }
    if (fd >= 0)
        (void) close(fd);
    if (fj) {
        (void) unlink(jname);
        fj = 0;
60        offset = 0;
    }
}

```

**Table 1 (cont')**

	/*		
	* write a filled jmp struct offset of the prev one (if any): nw()		
	*/		
5	writejumps(ix)		writejumps
	int ix;		
	{		
	char *mktemp();		
10	if (!fj) {		
	if (mktemp(jname) < 0) {		
	fprintf(stderr, "%s: can't mktemp() %s\n", prog, jname);		
	cleanup(1);		
	}		
15	if ((fj = fopen(jname, "w")) == 0) {		
	fprintf(stderr, "%s: can't write %s\n", prog, jname);		
	exit(1);		
	}		
20	(void) fwrite((char *)&dx[ix].jp, sizeof(struct jmp), 1, fj);		
	(void) fwrite((char *)&dx[ix].offset, sizeof(dx[ix].offset), 1, fj);		
	}		
25			
30			
35			
40			
45			
50			
55			
60			



[illegible]

PRO	XXXXXXXXXXXXXXXXX	(Length = 15 amino acids)
Comparison Protein	XXXXXXXXYYYYYYY	(Length = 12 amino acids)

5      % amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =

10      5 divided by 15 = 33.3%

**Table 3**

PRO	XXXXXXXXXX	(Length = 10 amino acids)
Comparison Protein	XXXXXYYYYYYYZZYZ	(Length = 15 amino acids)

5      % amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =

10      5 divided by 10 = 50%

**Table 4**

PRO-DNA	NNNNNNNNNNNNNNNN	(Length = 14 nucleotides)
Comparison DNA	NNNNNNLLLLLLLLLLLL	(Length = 16 nucleotides)

5      % nucleic acid sequence identity =

(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =

10      6 divided by 14 = 42.9%

**Table 5**

PRO-DNA	NNNNNNNNNNNN	(Length = 12 nucleotides)
Comparison DNA	NNNNLLLLVV	(Length = 9 nucleotides)

5      % nucleic acid sequence identity =

(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =

10      4 divided by 12 = 33.3%



**4. Full-length PRO185 Polypeptides**

The DNA28503 clone was isolated from a human tissue library. As far as is known, the DNA28503 sequence encodes a novel factor designated herein as PRO185. Although, using WU-BLAST2 sequence alignment computer programs, some sequence identities with known proteins were revealed.

**5. Full-length PRO210 and PRO217 Polypeptides**

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO210 and PRO217. In particular, Applicants have identified and isolated cDNAs encoding a PRO210 and PRO217 polypeptide, as disclosed in further detail in the Examples below. Using BLAST (FastA format) sequence alignment computer programs, Applicants found that cDNAs sequence encoding full-length native sequence PRO210 and PRO217 have homologies to known proteins having EGF-like domains. Accordingly, it is presently believed that the PRO210 and PRO217 polypeptides disclosed in the present application is a newly identified member of the EGF-like family and possesses properties typical of the EGF-like protein family.

**6. Full-length PRO215 Polypeptides**

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO215. In particular, Applicants have identified and isolated cDNAs encoding a PRO215 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that a cDNA sequence encoding full-length native sequence PRO215 (shown in Figure 11 and SEQ ID NO:16) encodes for a polypeptide having an amino acid sequence which has identity with the amino acid sequence of the SLIT protein precursor. PRO215 also has identity with a leucine rich repeat protein.

**7. Full-length PRO242 Polypeptides**

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO242. In particular, Applicants have identified and isolated cDNA encoding a PRO242 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that a cDNA sequence encoding full-length native sequence PRO242 (shown in Figure 15 and SEQ ID NO:23) has amino acid sequence identity with human macrophage inflammatory protein 1-alpha, rabbit macrophage inflammatory protein 1-beta, human LD78 and rabbit immune activation gene 2. Accordingly, it is presently believed that PRO242 polypeptide disclosed in the present application is a newly identified member of the chemokine family and possesses activity typical of the chemokine family.

**8. Full-length PRO288 Polypeptides**

The present invention provides newly identified and isolated PRO288 polypeptides. In particular, Applicants have identified and isolated various human PRO288 polypeptides. The properties and

characteristics of some of these PRO288 polypeptides are described in further detail in the Examples below. Based upon the properties and characteristics of the PRO288 polypeptides disclosed herein, it is Applicants' present belief that PRO288 is a member of the TNFR family, and particularly, is a receptor for Apo-2 ligand.

**9. Full-length PRO365 Polypeptides**

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO365. In particular, Applicants have identified and isolated cDNA encoding a PRO365 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that various portions of the PRO365 polypeptide have significant homology with the human 2-19 protein. Accordingly, it is presently believed that PRO365 polypeptide disclosed in the present application is a newly identified member of the human 2-19 protein family.

**10. Full-length PRO1361 Polypeptides**

The DNA60783-1611 clone was isolated from a human B cell library. As far as is known, the DNA60783-1611 sequence encodes a novel factor designated herein as PRO1361; using the WU-BLAST2 sequence alignment computer program, no sequence identities to any known proteins were revealed.

**11. Full-length PRO1308 Polypeptides**

Using WU-BLAST2 sequence alignment computer programs, it has been found that PRO1308 shares certain amino acid sequence identity with the amino acid sequence of the follistatin protein designated "S55369" in the Dayhoff database. Accordingly, it is presently believed that PRO1308 disclosed in the present application is a newly identified member of the follistatin protein family and may possess activity or properties typical of that family of proteins.

**12. Full-length PRO1183 Polypeptides**

Using WU-BLAST2 sequence alignment computer programs, it has been found that a full-length native sequence PRO1183 (shown in Figure 26 and SEQ ID NO:52) has certain amino acid sequence identity with protoporphyrinogen oxidase. Accordingly, it is presently believed that PRO1183 disclosed in the present application is a newly identified member of the oxidase family and may possess enzymatic activity typical of oxidases.

**13. Full-length PRO1272 Polypeptides**

Using WU-BLAST2 sequence alignment computer programs, it has been found that a full-length native sequence PRO1272 (shown in Figure 28 and SEQ ID NO:54) has certain amino acid sequence identity with cement gland-specific protein from *Xenopus laevis*. Accordingly, it is presently believed that PRO1272 disclosed in the present application is a newly identified member of the XAG family and may share at least one mechanism with the XAG proteins.

**14. Full-length PRO1419 Polypeptides**

As far as is known, the DNA71290-1630 sequence encodes a novel factor designated herein as PRO1419. Using WU-BLAST2 sequence alignment computer programs, minimal sequence identities to known proteins were revealed.

**15. Full-length PRO4999 Polypeptides**

Using the ALIGN-2 sequence alignment computer program referenced above, it has been found that the full-length native sequence PRO4999 (shown in Figure 32 and SEQ ID NO:58) has certain amino acid sequence identity with UROM\_HUMAN. Accordingly, it is presently believed that the PRO4999 polypeptide disclosed in the present application is a newly identified member of the uromodulin protein family and may possess one or more biological and/or immunological activities or properties typical of that protein family.

**16. Full-length PRO7170 Polypeptides**

The DNA108722-2743 clone was isolated from a human library as described in the Examples below. As far as is known, the DNA108722-2743 nucleotide sequence encodes a novel factor designated herein as PRO7170; using the ALIGN-2 sequence alignment computer program, no significant sequence identities to any known proteins were revealed.

**17. Full-length PRO248 Polypeptides**

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO248. In particular, Applicants have identified and isolated cDNA encoding a PRO248 polypeptide, as disclosed in further detail in the Examples below. Using known programs such as BLAST and FastA sequence alignment computer programs, Applicants found that a cDNA sequence encoding full-length native sequence PRO248 (amino acid sequence shown in Figure 36 and SEQ ID NO:65) has certain amino acid sequence identity with growth differentiation factor 3, from mouse and from homo sapiens. Accordingly, it is presently believed that PRO248 polypeptide disclosed in the present application is a newly identified member of the transforming growth factor  $\beta$  family and possesses growth and differentiation capabilities typical of the this family.

**18. Full-length PRO353 Polypeptides**

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO353. In particular, Applicants have identified and isolated cDNA encoding PRO353 polypeptides, as disclosed in further detail in the Examples below. Using BLAST and, FastA sequence alignment computer programs, Applicants found that various portions of the PRO353 polypeptides have certain homology with the human and mouse complement proteins. Accordingly, it is presently believed that the PRO353 polypeptides disclosed in the present application are newly identified members of the complement protein family and possesses the ability to effect the inflammation process as is typical of the complement family of proteins.



**19. Full-length PRO1318 and PRO1600 Polypeptides**

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO1318 and PRO1600. In particular, Applicants have identified and isolated cDNAs encoding PRO1318 and PRO1600 polypeptides, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that cDNA sequence encoding full-length native sequence PRO1318 and PRO1600 (shown in Figure 40 and SEQ ID NO:78 and Figure 42 and SEQ ID NO:80, respectively) have amino acid sequence identity with one or more chemokines. Accordingly, it is presently believed that the PRO1318 and PRO1600 polypeptides disclosed in the present application are newly identified members of the chemokine family and possesses activity typical of the chemokine family.

**20. Full-length PRO533 Polypeptides**

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO533. In particular, Applicants have identified and isolated cDNA encoding a PRO533 polypeptide, as disclosed in further detail in the Examples below. Using BLAST-2 and FastA sequence alignment computer programs, Applicants found that a full-length native sequence PRO533 (shown in Figure 46 and SEQ ID NO:86) has a Blast score of 509 and 53% amino acid sequence identity with fibroblast growth factor (FGF). Accordingly, it is presently believed that PRO533 disclosed in the present application is a newly identified member of the fibroblast growth factor family and may possess activity typical of such polypeptides.

**21. Full-length PRO301 Polypeptides**

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO301. In particular, Applicants have identified and isolated cDNA encoding a PRO301 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that a full-length native sequence PRO301 (shown in Figure 48 and SEQ ID NO:91) has a Blast score of 246 corresponding to 30% amino acid sequence identity with human A33 antigen precursor. Accordingly, it is presently believed that PRO301 disclosed in the present application is a newly identified member of the A33 antigen protein family and may be expressed in human neoplastic diseases such as colorectal cancer.

**22. Full-length PRO187 Polypeptides**

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO187. In particular, Applicants have identified and isolated cDNA encoding a PRO187 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that a full-length native sequence PRO187 (shown in Figure 50) has 74% amino acid sequence identity and BLAST score of 310 with various androgen-induced growth factors and FGF-8. Accordingly, it is presently believed that PRO187 polypeptide

disclosed in the present application is a newly identified member of the FGF-8 protein family and may possess identify activity or property typical of the FGF-8-like protein family.

### 23. Full-length PRO337 Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO337. In particular, Applicants have identified and isolated cDNA encoding a PRO337 polypeptide, as disclosed in further detail in the Examples below. Using BLAST, BLAST-2 and FastA sequence alignment computer programs, Applicants found that a full-length native sequence PRO337 has 97% amino acid sequence identity with rat neurotrimin, 85% sequence identity with chicken CEPU, 73% sequence identity with chicken G55, 59% homology with human LAMP and 84% homology with human OPCAM. Accordingly, it is presently believed that PRO337 disclosed in the present application is a newly identified member of the IgLON sub family of the immunoglobulin superfamily and may possess neurite growth and differentiation potentiating properties.

### 24. Full-length PRO1411 Polypeptides

As far as is known, the DNA59212-1627 sequence encodes a novel factor designated herein as PRO1411. However, using WU-BLAST2 sequence alignment computer programs, some sequence identities to known proteins were revealed.

### 25. Full-length PRO4356 Polypeptides

Using WU-BLAST2 sequence alignment computer programs, it has been found that a full-length native sequence PRO4356 (shown in Figure 56 and SEQ ID NO:108) has certain amino acid sequence identity with metastasis associated GPI-anchored protein. Accordingly, it is presently believed that PRO4356 disclosed in the present application is a newly identified member of this family and shares similar mechanisms.

### 26. Full-length PRO246 Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO246. In particular, Applicants have identified and isolated cDNA encoding a PRO246 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that a portion of the PRO246 polypeptide has significant homology with the human cell surface protein HCAR. Accordingly, it is presently believed that PRO246 polypeptide disclosed in the present application may be a newly identified membrane-bound virus receptor or tumor cell-specific antigen.

### 27. Full-length PRO265 Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO265. In particular, Applicants have identified and isolated cDNA encoding a PRO265 polypeptide, as disclosed in further detail in the Examples below. Using

programs such as BLAST and FastA sequence alignment computer programs, Applicants found that various portions of the PRO265 polypeptide have significant homology with the fibromodulin protein and fibromodulin precursor protein. Applicants have also found that the DNA encoding the PRO265 polypeptide has significant homology with platelet glycoprotein V, a member of the leucine rich related protein family involved in skin and wound repair. Accordingly, it is presently believed that PRO265 polypeptide disclosed in the present application is a newly identified member of the leucine rich repeat family and possesses protein protein binding capabilities, as well as be involved in skin and wound repair as typical of this family.

#### 28. Full-length PRO941 Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO941. In particular, Applicants have identified and isolated cDNA encoding a PRO941 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that the PRO941 polypeptide has significant similarity to one or more cadherin proteins. Accordingly, it is presently believed that PRO941 polypeptide disclosed in the present application is a newly identified cadherin homolog.

#### 29. Full-length PRO10096 Polypeptides

Using the ALIGN-2 sequence alignment computer program referenced above, it has been found that the full-length native sequence PRO10096 (shown in Figure 64 and SEQ ID NO: 126) has certain amino acid sequence identity with various interleukin-10-related molecules. Accordingly, it is presently believed that the PRO10096 polypeptide disclosed in the present application is a newly identified IL-10 homolog and may possess one or more biological and/or immunological activities or properties typical of that protein.

#### 30. Full-length PRO6003 Polypeptides

The DNA83568-2692 clone was isolated from a human fetal kidney library as described in the Examples below. As far as is known, the DNA83568-2692 nucleotide sequence encodes a novel factor designated herein as PRO6003; using the ALIGN-2 sequence alignment computer program, no significant sequence identities to any known proteins were revealed.

#### B. PRO Polypeptide Variants

In addition to the full-length native sequence PRO polypeptides described herein, it is contemplated that PRO variants can be prepared. PRO variants can be prepared by introducing appropriate nucleotide changes into the PRO DNA, and/or by synthesis of the desired PRO polypeptide. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of the PRO, such as changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

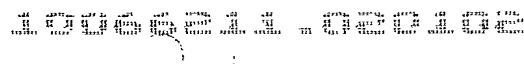
Variations in the native full-length sequence PRO or in various domains of the PRO described herein, can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent No. 5,364,934. Variations may be a substitution, deletion or

insertion of one or more codons encoding the PRO that results in a change in the amino acid sequence of the PRO as compared with the native sequence PRO. Optionally the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of the PRO. Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the PRO with that of homologous known protein molecules and minimizing the number of amino acid sequence changes made in regions of high homology. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, i.e., conservative amino acid replacements. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the full-length or mature native sequence.

PRO polypeptide fragments are provided herein. Such fragments may be truncated at the N-terminus or C-terminus, or may lack internal residues, for example, when compared with a full length native protein. Certain fragments lack amino acid residues that are not essential for a desired biological activity of the PRO polypeptide.

PRO fragments may be prepared by any of a number of conventional techniques. Desired peptide fragments may be chemically synthesized. An alternative approach involves generating PRO fragments by enzymatic digestion, e.g., by treating the protein with an enzyme known to cleave proteins at sites defined by particular amino acid residues, or by digesting the DNA with suitable restriction enzymes and isolating the desired fragment. Yet another suitable technique involves isolating and amplifying a DNA fragment encoding a desired polypeptide fragment, by polymerase chain reaction (PCR). Oligonucleotides that define the desired termini of the DNA fragment are employed at the 5' and 3' primers in the PCR. Preferably, PRO polypeptide fragments share at least one biological and/or immunological activity with the native PRO polypeptide disclosed herein.

In particular embodiments, conservative substitutions of interest are shown in Table 6 under the heading of preferred substitutions. If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 6, or as further described below in reference to amino acid classes, are introduced and the products screened.



**Table 6**

	<u>Original Residue</u>	<u>Exemplary Substitutions</u>	<u>Preferred Substitutions</u>
5	Ala (A)	val; leu; ile	val
	Arg (R)	lys; gln; asn	lys
	Asn (N)	gln; his; lys; arg	gln
	Asp (D)	glu	glu
	Cys (C)	ser	ser
10	Gln (Q)	asn	asn
	Glu (E)	asp	asp
	Gly (G)	pro; ala	ala
	His (H)	asn; gln; lys; arg	arg
	Ile (I)	leu; val; met; ala; phe;	
15		norleucine	leu
	Leu (L)	norleucine; ile; val;	
		met; ala; phe	ile
	Lys (K)	arg; gln; asn	arg
	Met (M)	leu; phe; ile	leu
20	Phe (F)	leu; val; ile; ala; tyr	leu
	Pro (P)	ala	ala
	Ser (S)	thr	thr
	Thr (T)	ser	ser
	Trp (W)	tyr; phe	tyr
25	Tyr (Y)	trp; phe; thr; ser	phe
	Val (V)	ile; leu; met; phe;	
		ala; norleucine	leu

30 Substantial modifications in function or immunological identity of the PRO polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

- 35 (1) hydrophobic: norleucine, met, ala, val, leu, ile;  
 (2) neutral hydrophilic: cys, ser, thr;  
 (3) acidic: asp, glu;  
 (4) basic: asn, gln, his, lys, arg;  
 (5) residues that influence chain orientation: gly, pro; and  
 (6) aromatic: trp, tyr, phe.

40 Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, more preferably, into the remaining (non-conserved) sites.

45 The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter et al., Nucl. Acids Res., 13:4331 (1986); Zoller et al., Nucl. Acids Res., 10:6487 (1987)], cassette mutagenesis [Wells et al., Gene, 34:315 (1985)], restriction selection mutagenesis [Wells et al., Philos. Trans. R. Soc. London SerA,

317:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the PRO variant DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant [Cunningham and Wells, Science, 244: 1081-1085 (1989)]. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, The Proteins, (W.H. Freeman & Co., N.Y.); Chothia, J. Mol. Biol., 150:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

### C. Modifications of PRO

Covalent modifications of PRO are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of a PRO polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C- terminal residues of the PRO. Derivatization with bifunctional agents is useful, for instance, for crosslinking PRO to a water-insoluble support matrix or surface for use in the method for purifying anti-PRO antibodies, and vice-versa. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)dithio]propioimide.

Other modifications include deamidation of glutamyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the  $\alpha$ -amino groups of lysine, arginine, and histidine side chains [T.E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the PRO polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in native sequence PRO (either by removing the underlying glycosylation site or by deleting the glycosylation by chemical and/or enzymatic means), and/or adding one or more glycosylation sites that are not present in the native sequence PRO. In addition, the phrase includes qualitative changes in the glycosylation of the native proteins, involving a change in the nature and proportions of the various carbohydrate moieties present.

Addition of glycosylation sites to the PRO polypeptide may be accomplished by altering the amino acid sequence. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to the native sequence PRO (for O-linked glycosylation sites). The PRO amino

acid sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the PRO polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

Another means of increasing the number of carbohydrate moieties on the PRO polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, e.g., in WO 87/05330 published 11 September 1987, and in Aplin and Wriston, CRC Crit. Rev. Biochem., pp. 259-306 (1981).

Removal of carbohydrate moieties present on the PRO polypeptide may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are known in the art and described, for instance, by Hakimuddin, et al., Arch. Biochem. Biophys., 259:52 (1987) and by Edge et al., Anal. Biochem., 118:131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al., Meth. Enzymol., 138:350 (1987).

Another type of covalent modification of PRO comprises linking the PRO polypeptide to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol (PEG), polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

The PRO of the present invention may also be modified in a way to form a chimeric molecule comprising PRO fused to another, heterologous polypeptide or amino acid sequence.

In one embodiment, such a chimeric molecule comprises a fusion of the PRO with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl- terminus of the PRO. The presence of such epitope-tagged forms of the PRO can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the PRO to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field et al., Mol. Cell. Biol., 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al., Molecular and Cellular Biology, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et al., Protein Engineering, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp et al., BioTechnology, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin et al., Science, 255:192-194 (1992)]; an  $\alpha$ -tubulin epitope peptide [Skinner et al., J. Biol. Chem., 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., Proc. Natl. Acad. Sci. USA, 87:6393-6397 (1990)].

In an alternative embodiment, the chimeric molecule may comprise a fusion of the PRO with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule (also referred to as an "immunoadhesin"), such a fusion could be to the Fc region of an IgG molecule. The Ig fusions preferably include the substitution of a soluble (transmembrane domain deleted or inactivated) form of a PRO polypeptide in place of at least one variable region within an Ig molecule. In a particularly preferred

embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3, or the hinge, CH1, CH2 and CH3 regions of an IgG1 molecule. For the production of immunoglobulin fusions see also US Patent No. 5,428,130 issued June 27, 1995.

#### D. Preparation of PRO

The description below relates primarily to production of PRO by culturing cells transformed or transfected with a vector containing PRO nucleic acid. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare PRO. For instance, the PRO sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques [see, e.g., Stewart et al., Solid-Phase Peptide Synthesis, W.H. Freeman Co., San Francisco, CA (1969); Merrifield, J. Am. Chem. Soc., **85**:2149-2154 (1963)]. *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of the PRO may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the full-length PRO.

##### 1. Isolation of DNA Encoding PRO

DNA encoding PRO may be obtained from a cDNA library prepared from tissue believed to possess the PRO mRNA and to express it at a detectable level. Accordingly, human PRO DNA can be conveniently obtained from a cDNA library prepared from human tissue, such as described in the Examples. The PRO-encoding gene may also be obtained from a genomic library or by known synthetic procedures (e.g., automated nucleic acid synthesis).

Libraries can be screened with probes (such as antibodies to the PRO or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook et al., Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene encoding PRO is to use PCR methodology [Sambrook et al., *supra*; Dieffenbach et al., PCR Primer: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1995)].

The Examples below describe techniques for screening a cDNA library. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like <sup>32</sup>P-labeled ATP, biotinylation or enzyme labeling. Hybridization conditions, including moderate stringency and high stringency, are provided in Sambrook et al., *supra*.

Sequences identified in such library screening methods can be compared and aligned to other known sequences deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined using methods known in the art and as described herein.



Nucleic acid having protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in Sambrook et al., supra, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

## 2. Selection and Transformation of Host Cells

Host cells are transfected or transformed with expression or cloning vectors described herein for PRO production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in Mammalian Cell Biotechnology: a Practical Approach, M. Butler, ed. (IRL Press, 1991) and Sambrook et al., supra.

Methods of eukaryotic cell transfection and prokaryotic cell transformation are known to the ordinarily skilled artisan, for example,  $\text{CaCl}_2$ ,  $\text{CaPO}_4$ , liposome-mediated and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook et al., supra, or electroporation is generally used for prokaryotes. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw et al., Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) can be employed. General aspects of mammalian cell host system transfections have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen et al., J. Bact., 130:946 (1977) and Hsiao et al., Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, e.g., polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown et al., Methods in Enzymology, 185:527-537 (1990) and Mansour et al., Nature, 336:348-352 (1988).

Suitable host cells for cloning or expressing the DNA in the vectors herein include prokaryote, yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *E. coli*. Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635). Other suitable prokaryotic host cells include Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. These examples are illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic

enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding proteins endogenous to the host, with examples of such hosts including *E. coli* W3110 strain 1A2, which has the complete genotype *tonA*; *E. coli* W3110 strain 9E4, which has the complete genotype *tonA ptr3*; *E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT kan'*; *E. coli* W3110 strain 37D6, which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT rbs7 ilvG kan'*; *E. coli* W3110 strain 40B4, which is strain 37D6 with a non-kanamycin resistant *degP* deletion mutation; and an *E. coli* strain having mutant periplasmic protease disclosed in U.S. Patent No. 4,946,783 issued 7 August 1990. Alternatively, *in vitro* methods of cloning, e.g., PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for PRO-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe* (Beach and Nurse, *Nature*, 290: 140 [1981]; EP 139,383 published 2 May 1985); *Kluyveromyces* hosts (U.S. Patent No. 4,943,529; Fleer et al., *Bio/Technology*, 9:968-975 (1991)) such as, e.g., *K. lactis* (MW98-8C, CBS683, CBS4574; Louvencourt et al., *J. Bacteriol.*, 154(2):737-742 [1983]), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilum* (ATCC 36,906; Van den Berg et al., *Bio/Technology*, 8:135 (1990)), *K. thermotolerans*, and *K. marxianus*; *yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070; Sreekrishna et al., *J. Basic Microbiol.*, 28:265-278 [1988]); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa* (Case et al., *Proc. Natl. Acad. Sci. USA*, 76:5259-5263 [1979]); *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 October 1990); and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium* (WO 91/00357 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans* (Ballance et al., *Biochem. Biophys. Res. Commun.*, 112:284-289 [1983]; Tilburn et al., *Gene*, 26:205-221 [1983]; Yelton et al., *Proc. Natl. Acad. Sci. USA*, 81: 1470-1474 [1984]) and *A. niger* (Kelly and Hynes, *EMBO J.*, 4:475-479 [1985]). Methylophilic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on methanol selected from the genera consisting of *Hansenula*, *Candida*, *Kloeckera*, *Pichia*, *Saccharomyces*, *Torulopsis*, and *Rhodotorula*. A list of specific species that are exemplary of this class of yeasts may be found in C. Anthony, *The Biochemistry of Methylophilic Yeasts*, 269 (1982).

Suitable host cells for the expression of glycosylated PRO are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as *Drosophila* S2 and *Spodoptera* Sf9, as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., *J. Gen Virol.*, 36:59 (1977)); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.*, 23:243-251 (1980)); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor (MMT 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

### 3. Selection and Use of a Replicable Vector

The nucleic acid (e.g., cDNA or genomic DNA) encoding PRO may be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

The PRO may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the PRO-encoding DNA that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, e.g., the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces*  $\alpha$ -factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the  $2\mu$  plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells.

Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*.

An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the PRO-encoding nucleic acid, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR-activity, prepared and propagated as described by Urlaub et al., Proc. Natl. Acad. Sci. USA, 77:4216 (1980). A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 [Stinchcomb et al., Nature, 282:39 (1979); Kingsman et al., Gene, 7:141 (1979); Tschemper et al., Gene, 10:157 (1980)]. The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan,

for example, ATCC No. 44076 or PEP4-1 [Jones, Genetics, 85:12 (1977)].

Expression and cloning vectors usually contain a promoter operably linked to the PRO-encoding nucleic acid sequence to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the  $\beta$ -lactamase and lactose promoter systems [Chang et al., Nature, 275:615 (1978); Goeddel et al., Nature, 281:544 (1979)], alkaline phosphatase, a tryptophan (trp) promoter system [Goeddel, Nucleic Acids Res., 8:4057 (1980); EP 36,776], and hybrid promoters such as the tac promoter [deBoer et al., Proc. Natl. Acad. Sci. USA, 80:21-25 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding PRO.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman et al., J. Biol. Chem., 255:2073 (1980)] or other glycolytic enzymes [Hess et al., J. Adv. Enzyme Reg., 7:149 (1968); Holland, Biochemistry, 17:4900 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657.

PRO transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with the host cell systems.

Transcription of a DNA encoding the PRO by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin,  $\alpha$ -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the PRO coding sequence, but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide

segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding PRO.

Still other methods, vectors, and host cells suitable for adaptation to the synthesis of PRO in recombinant vertebrate cell culture are described in Gething et al., Nature, 293:620-625 (1981); Mantei et al., Nature, 281:40-46 (1979); EP 117,060; and EP 117,058.

#### 4. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 (1980)], dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence PRO polypeptide or against a synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to PRO DNA and encoding a specific antibody epitope.

#### 5. Purification of Polypeptide

Forms of PRO may be recovered from culture medium or from host cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (e.g. Triton-X 100) or by enzymatic cleavage. Cells employed in expression of PRO can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

It may be desired to purify PRO from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the PRO. Various methods of protein purification may be employed and such methods are known in the art and described for example in Deutscher, Methods in Enzymology, 182 (1990); Scopes, Protein Purification: Principles and Practice, Springer-Verlag, New York (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular PRO produced.

#### E. Uses for PRO

Nucleotide sequences (or their complement) encoding PRO have various applications in the art of molecular biology, including uses as hybridization probes, in chromosome and gene mapping and in the generation of anti-sense RNA and DNA. PRO nucleic acid will also be useful for the preparation of PRO polypeptides by the recombinant techniques described herein.

5 The full-length native sequence PRO gene, or portions thereof, may be used as hybridization probes for a cDNA library to isolate the full-length PRO cDNA or to isolate still other cDNAs (for instance, those encoding naturally-occurring variants of PRO or PRO from other species) which have a desired sequence identity to the native PRO sequence disclosed herein. Optionally, the length of the probes will be about 20 to about 50 bases. The hybridization probes may be derived from at least partially novel regions of the full length  
10 native nucleotide sequence wherein those regions may be determined without undue experimentation or from genomic sequences including promoters, enhancer elements and introns of native sequence PRO. By way of example, a screening method will comprise isolating the coding region of the PRO gene using the known DNA sequence to synthesize a selected probe of about 40 bases. Hybridization probes may be labeled by a variety of labels, including radionucleotides such as <sup>32</sup>P or <sup>35</sup>S, or enzymatic labels such as alkaline phosphatase  
15 coupled to the probe via avidin/biotin coupling systems. Labeled probes having a sequence complementary to that of the PRO gene of the present invention can be used to screen libraries of human cDNA, genomic DNA or mRNA to determine which members of such libraries the probe hybridizes to. Hybridization techniques are described in further detail in the Examples below.

20 Any EST sequences disclosed in the present application may similarly be employed as probes, using the methods disclosed herein.

Other useful fragments of the PRO nucleic acids include antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target PRO mRNA (sense) or PRO DNA (antisense) sequences. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment of the coding region of PRO DNA. Such a fragment generally  
25 comprises at least about 14 nucleotides, preferably from about 14 to 30 nucleotides. The ability to derive an antisense or a sense oligonucleotide, based upon a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen (Cancer Res. 48:2659, 1988) and van der Krol et al. (BioTechniques 6:958, 1988).

Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation  
30 of duplexes that block transcription or translation of the target sequence by one of several means, including enhanced degradation of the duplexes, premature termination of transcription or translation, or by other means. The antisense oligonucleotides thus may be used to block expression of PRO proteins. Antisense or sense oligonucleotides further comprise oligonucleotides having modified sugar-phosphodiester backbones (or other sugar linkages, such as those described in WO 91/06629) and wherein such sugar linkages are resistant to  
35 endogenous nucleases. Such oligonucleotides with resistant sugar linkages are stable *in vivo* (i.e., capable of resisting enzymatic degradation) but retain sequence specificity to be able to bind to target nucleotide sequences.

Other examples of sense or antisense oligonucleotides include those oligonucleotides which are covalently linked to organic moieties, such as those described in WO 90/10048, and other moieties that increases affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L-lysine). Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense oligonucleotides to modify binding specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.

Antisense or sense oligonucleotides may be introduced into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example,  $\text{CaPO}_4$ -mediated DNA transfection, electroporation, or by using gene transfer vectors such as Epstein-Barr virus. In a preferred procedure, an antisense or sense oligonucleotide is inserted into a suitable retroviral vector. A cell containing the target nucleic acid sequence is contacted with the recombinant retroviral vector, either *in vivo* or *ex vivo*. Suitable retroviral vectors include, but are not limited to, those derived from the murine retrovirus M-MuLV, N2 (a retrovirus derived from M-MuLV), or the double copy vectors designated DCT5A, DCT5B and DCT5C (see WO 90/13641).

Sense or antisense oligonucleotides also may be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, as described in WO 91/04753. Suitable ligand binding molecules include, but are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands that bind to cell surface receptors. Preferably, conjugation of the ligand binding molecule does not substantially interfere with the ability of the ligand binding molecule to bind to its corresponding molecule or receptor, or block entry of the sense or antisense oligonucleotide or its conjugated version into the cell.

Alternatively, a sense or an antisense oligonucleotide may be introduced into a cell containing the target nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or antisense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase.

Antisense or sense RNA or DNA molecules are generally at least about 5 bases in length, about 10 bases in length, about 15 bases in length, about 20 bases in length, about 25 bases in length, about 30 bases in length, about 35 bases in length, about 40 bases in length, about 45 bases in length, about 50 bases in length, about 55 bases in length, about 60 bases in length, about 65 bases in length, about 70 bases in length, about 75 bases in length, about 80 bases in length, about 85 bases in length, about 90 bases in length, about 95 bases in length, about 100 bases in length, or more.

The probes may also be employed in PCR techniques to generate a pool of sequences for identification of closely related PRO coding sequences.

Nucleotide sequences encoding a PRO can also be used to construct hybridization probes for mapping the gene which encodes that PRO and for the genetic analysis of individuals with genetic disorders. The nucleotide sequences provided herein may be mapped to a chromosome and specific regions of a chromosome using known techniques, such as *in situ* hybridization, linkage analysis against known chromosomal markers, and hybridization screening with libraries.

When the coding sequences for PRO encode a protein which binds to another protein (example, where

the PRO is a receptor), the PRO can be used in assays to identify the other proteins or molecules involved in the binding interaction. By such methods, inhibitors of the receptor/ligand binding interaction can be identified. Proteins involved in such binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction. Also, the receptor PRO can be used to isolate correlative ligand(s). Screening assays can be designed to find lead compounds that mimic the biological activity of a native PRO or a receptor for PRO. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated include synthetic organic or inorganic compounds. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art.

Nucleic acids which encode PRO or its modified forms can also be used to generate either transgenic animals or "knock out" animals which, in turn, are useful in the development and screening of therapeutically useful reagents. A transgenic animal (e.g., a mouse or rat) is an animal having cells that contain a transgene, which transgene was introduced into the animal or an ancestor of the animal at a prenatal, e.g., an embryonic stage. A transgene is a DNA which is integrated into the genome of a cell from which a transgenic animal develops. In one embodiment, cDNA encoding PRO can be used to clone genomic DNA encoding PRO in accordance with established techniques and the genomic sequences used to generate transgenic animals that contain cells which express DNA encoding PRO. Methods for generating transgenic animals, particularly animals such as mice or rats, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009. Typically, particular cells would be targeted for PRO transgene incorporation with tissue-specific enhancers. Transgenic animals that include a copy of a transgene encoding PRO introduced into the germ line of the animal at an embryonic stage can be used to examine the effect of increased expression of DNA encoding PRO. Such animals can be used as tester animals for reagents thought to confer protection from, for example, pathological conditions associated with its overexpression. In accordance with this facet of the invention, an animal is treated with the reagent and a reduced incidence of the pathological condition, compared to untreated animals bearing the transgene, would indicate a potential therapeutic intervention for the pathological condition.

Alternatively, non-human homologues of PRO can be used to construct a PRO "knock out" animal which has a defective or altered gene encoding PRO as a result of homologous recombination between the endogenous gene encoding PRO and altered genomic DNA encoding PRO introduced into an embryonic stem cell of the animal. For example, cDNA encoding PRO can be used to clone genomic DNA encoding PRO in accordance with established techniques. A portion of the genomic DNA encoding PRO can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see e.g., Thomas and Capecchi, Cell, 51:503 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected [see e.g., Li et al., Cell, 69:915 (1992)]. The selected cells are then injected into a blastocyst of an animal



(e.g., a mouse or rat) to form aggregation chimeras [see e.g., Bradley, in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized for instance, for their ability to defend against certain pathological conditions and for their development of pathological conditions due to absence of the PRO polypeptide.

Nucleic acid encoding the PRO polypeptides may also be used in gene therapy. In gene therapy applications, genes are introduced into cells in order to achieve *in vivo* synthesis of a therapeutically effective genetic product, for example for replacement of a defective gene. "Gene therapy" includes both conventional gene therapy where a lasting effect is achieved by a single treatment, and the administration of gene therapeutic agents, which involves the one time or repeated administration of a therapeutically effective DNA or mRNA. Antisense RNAs and DNAs can be used as therapeutic agents for blocking the expression of certain genes *in vivo*. It has already been shown that short antisense oligonucleotides can be imported into cells where they act as inhibitors, despite their low intracellular concentrations caused by their restricted uptake by the cell membrane. (Zamecnik *et al.*, Proc. Natl. Acad. Sci. USA 83:4143-4146 [1986]). The oligonucleotides can be modified to enhance their uptake, e.g. by substituting their negatively charged phosphodiester groups by uncharged groups.

There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro*, or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. The currently preferred *in vivo* gene transfer techniques include transfection with viral (typically retroviral) vectors and viral coat protein-liposome mediated transfection (Dzau *et al.*, Trends in Biotechnology 11, 205-210 [1993]). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cell, etc. Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, e.g. capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu *et al.*, J. Biol. Chem. 262, 4429-4432 (1987); and Wagner *et al.*, Proc. Natl. Acad. Sci. USA 87, 3410-3414 (1990). For review of gene marking and gene therapy protocols see Anderson *et al.*, Science 256, 808-813 (1992).

The PRO polypeptides described herein may also be employed as molecular weight markers for protein electrophoresis purposes and the isolated nucleic acid sequences may be used for recombinantly expressing those markers.

The nucleic acid molecules encoding the PRO polypeptides or fragments thereof described herein are useful for chromosome identification. In this regard, there exists an ongoing need to identify new chromosome markers, since relatively few chromosome marking reagents, based upon actual sequence data are presently available. Each PRO nucleic acid molecule of the present invention can be used as a chromosome marker.

The PRO polypeptides and nucleic acid molecules of the present invention may also be used for tissue typing, wherein the PRO polypeptides of the present invention may be differentially expressed in one tissue as compared to another. PRO nucleic acid molecules will find use for generating probes for PCR, Northern analysis, Southern analysis and Western analysis.

The PRO polypeptides described herein may also be employed as therapeutic agents. The PRO polypeptides of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the PRO product hereof is combined in admixture with a pharmaceutically acceptable carrier vehicle. Therapeutic formulations are prepared for storage by mixing the active ingredient having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone, amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, PLURONICS™ or PEG.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution.

Therapeutic compositions herein generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The route of administration is in accord with known methods, e.g. injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial or intralesional routes, topical administration, or by sustained release systems.

Dosages and desired drug concentrations of pharmaceutical compositions of the present invention may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary physician. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The use of interspecies scaling in toxicokinetics" In *Toxicokinetics and New Drug Development*, Yacobi et al., Eds., Pergamon Press, New York 1989, pp. 42-96.

When *in vivo* administration of a PRO polypeptide or agonist or antagonist thereof is employed, normal dosage amounts may vary from about 10 ng/kg to up to 100 mg/kg of mammal body weight or more

per day, preferably about 1  $\mu$ g/kg/day to 10 mg/kg/day, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Pat. Nos. 4,657,760; 5,206,344; or 5,225,212. It is anticipated that different formulations will be effective for different treatment compounds and different disorders, that administration targeting one organ or tissue, for example, may necessitate delivery in a manner different from that to another organ or tissue.

Where sustained-release administration of a PRO polypeptide is desired in a formulation with release characteristics suitable for the treatment of any disease or disorder requiring administration of the PRO polypeptide, microencapsulation of the PRO polypeptide is contemplated. Microencapsulation of recombinant proteins for sustained release has been successfully performed with human growth hormone (rhGH), interferon- $\gamma$  (rhIFN- $\gamma$ ), interleukin-2, and MN rgp120. Johnson et al., Nat. Med., 2:795-799 (1996); Yasuda, Biomed. Ther., 27:1221-1223 (1993); Hora et al., Bio/Technology, 8:755-758 (1990); Cleland, "Design and Production of Single Immunization Vaccines Using Polylactide Polyglycolide Microsphere Systems," in Vaccine Design: The Subunit and Adjuvant Approach, Powell and Newman, eds. (Plenum Press: New York, 1995), pp. 439-462; WO 97/03692, WO 96/40072, WO 96/07399; and U.S. Pat. No. 5,654,010.

The sustained-release formulations of these proteins were developed using poly-lactic-co-glycolic acid (PLGA) polymer due to its biocompatibility and wide range of biodegradable properties. The degradation products of PLGA, lactic and glycolic acids, can be cleared quickly within the human body. Moreover, the degradability of this polymer can be adjusted from months to years depending on its molecular weight and composition. Lewis, "Controlled release of bioactive agents from lactide/glycolide polymer," in: M. Chasin and R. Langer (Eds.), Biodegradable Polymers as Drug Delivery Systems (Marcel Dekker: New York, 1990), pp. 1-41.

This invention encompasses methods of screening compounds to identify those that mimic the PRO polypeptide (agonists) or prevent the effect of the PRO polypeptide (antagonists). Screening assays for antagonist drug candidates are designed to identify compounds that bind or complex with the PRO polypeptides encoded by the genes identified herein, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates.

The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays, and cell-based assays, which are well characterized in the art.

All assays for antagonists are common in that they call for contacting the drug candidate with a PRO polypeptide encoded by a nucleic acid identified herein under conditions and for a time sufficient to allow these two components to interact.

In binding assays, the interaction is binding and the complex formed can be isolated or detected in the reaction mixture. In a particular embodiment, the PRO polypeptide encoded by the gene identified herein or the drug candidate is immobilized on a solid phase, e.g., on a microtiter plate, by covalent or non-covalent attachments. Non-covalent attachment generally is accomplished by coating the solid surface with a solution of the PRO polypeptide and drying. Alternatively, an immobilized antibody, e.g., a monoclonal antibody, specific for the PRO polypeptide to be immobilized can be used to anchor it to a solid surface. The assay is

performed by adding the non-immobilized component, which may be labeled by a detectable label, to the immobilized component, e.g., the coated surface containing the anchored component. When the reaction is complete, the non-reacted components are removed, e.g., by washing, and complexes anchored on the solid surface are detected. When the originally non-immobilized component carries a detectable label, the detection of label immobilized on the surface indicates that complexing occurred. Where the originally non-immobilized component does not carry a label, complexing can be detected, for example, by using a labeled antibody specifically binding the immobilized complex.

If the candidate compound interacts with but does not bind to a particular PRO polypeptide encoded by a gene identified herein, its interaction with that polypeptide can be assayed by methods well known for detecting protein-protein interactions. Such assays include traditional approaches, such as, e.g., cross-linking, co-immunoprecipitation, and co-purification through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored by using a yeast-based genetic system described by Fields and co-workers (Fields and Song, Nature (London), 340:245-246 (1989); Chien et al., Proc. Natl. Acad. Sci. USA, 88:9578-9582 (1991)) as disclosed by Chevray and Nathans, Proc. Natl. Acad. Sci. USA, 89: 5789-5793 (1991). Many transcriptional activators, such as yeast GAL4, consist of two physically discrete modular domains, one acting as the DNA-binding domain, the other one functioning as the transcription-activation domain. The yeast expression system described in the foregoing publications (generally referred to as the "two-hybrid system") takes advantage of this property, and employs two hybrid proteins, one in which the target protein is fused to the DNA-binding domain of GAL4, and another, in which candidate activating proteins are fused to the activation domain. The expression of a GAL1-*lacZ* reporter gene under control of a GAL4-activated promoter depends on reconstitution of GAL4 activity via protein-protein interaction. Colonies containing interacting polypeptides are detected with a chromogenic substrate for  $\beta$ -galactosidase. A complete kit (MATCHMAKER™) for identifying protein-protein interactions between two specific proteins using the two-hybrid technique is commercially available from Clontech. This system can also be extended to map protein domains involved in specific protein interactions as well as to pinpoint amino acid residues that are crucial for these interactions.

Compounds that interfere with the interaction of a gene encoding a PRO polypeptide identified herein and other intra- or extracellular components can be tested as follows: usually a reaction mixture is prepared containing the product of the gene and the intra- or extracellular component under conditions and for a time allowing for the interaction and binding of the two products. To test the ability of a candidate compound to inhibit binding, the reaction is run in the absence and in the presence of the test compound. In addition, a placebo may be added to a third reaction mixture, to serve as positive control. The binding (complex formation) between the test compound and the intra- or extracellular component present in the mixture is monitored as described hereinabove. The formation of a complex in the control reaction(s) but not in the reaction mixture containing the test compound indicates that the test compound interferes with the interaction of the test compound and its reaction partner.

To assay for antagonists, the PRO polypeptide may be added to a cell along with the compound to be screened for a particular activity and the ability of the compound to inhibit the activity of interest in the

presence of the PRO polypeptide indicates that the compound is an antagonist to the PRO polypeptide. Alternatively, antagonists may be detected by combining the PRO polypeptide and a potential antagonist with membrane-bound PRO polypeptide receptors or recombinant receptors under appropriate conditions for a competitive inhibition assay. The PRO polypeptide can be labeled, such as by radioactivity, such that the number of PRO polypeptide molecules bound to the receptor can be used to determine the effectiveness of the potential antagonist. The gene encoding the receptor can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting. Coligan et al., Current Protocols in Immun., 1(2): Chapter 5 (1991). Preferably, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the PRO polypeptide and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the PRO polypeptide. Transfected cells that are grown on glass slides are exposed to labeled PRO polypeptide. The PRO polypeptide can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase. Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an interactive sub-pooling and re-screening process, eventually yielding a single clone that encodes the putative receptor.

As an alternative approach for receptor identification, labeled PRO polypeptide can be photoaffinity-linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE and exposed to X-ray film. The labeled complex containing the receptor can be excised, resolved into peptide fragments, and subjected to protein micro-sequencing. The amino acid sequence obtained from micro-sequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the gene encoding the putative receptor.

In another assay for antagonists, mammalian cells or a membrane preparation expressing the receptor would be incubated with labeled PRO polypeptide in the presence of the candidate compound. The ability of the compound to enhance or block this interaction could then be measured.

More specific examples of potential antagonists include an oligonucleotide that binds to the fusions of immunoglobulin with PRO polypeptide, and, in particular, antibodies including, without limitation, polyclonal and monoclonal antibodies and antibody fragments, single-chain antibodies, anti-idiotypic antibodies, and chimeric or humanized versions of such antibodies or fragments, as well as human antibodies and antibody fragments. Alternatively, a potential antagonist may be a closely related protein, for example, a mutated form of the PRO polypeptide that recognizes the receptor but imparts no effect, thereby competitively inhibiting the action of the PRO polypeptide.

Another potential PRO polypeptide antagonist is an antisense RNA or DNA construct prepared using antisense technology, where, e.g., an antisense RNA or DNA molecule acts to block directly the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes the mature PRO polypeptides herein, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be

complementary to a region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res., 6:3073 (1979); Cooney et al., Science, 241: 456 (1988); Dervan et al., Science, 251:1360 (1991)), thereby preventing transcription and the production of the PRO polypeptide. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into the PRO polypeptide (antisense - Okano, Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression (CRC Press: Boca Raton, FL, 1988). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of the PRO polypeptide. When antisense DNA is used, oligodeoxyribonucleotides derived from the translation-initiation site, e.g., between about -10 and +10 positions of the target gene nucleotide sequence, are preferred.

Potential antagonists include small molecules that bind to the active site, the receptor binding site, or growth factor or other relevant binding site of the PRO polypeptide, thereby blocking the normal biological activity of the PRO polypeptide. Examples of small molecules include, but are not limited to, small peptides or peptide-like molecules, preferably soluble peptides, and synthetic non-peptidyl organic or inorganic compounds.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization to the complementary target RNA, followed by endonucleolytic cleavage. Specific ribozyme cleavage sites within a potential RNA target can be identified by known techniques. For further details see, e.g., Rossi, Current Biology, 4:469-471 (1994), and PCT publication No. WO 97/33551 (published September 18, 1997).

Nucleic acid molecules in triple-helix formation used to inhibit transcription should be single-stranded and composed of deoxynucleotides. The base composition of these oligonucleotides is designed such that it promotes triple-helix formation via Hoogsteen base-pairing rules, which generally require sizeable stretches of purines or pyrimidines on one strand of a duplex. For further details see, e.g., PCT publication No. WO 97/33551, *supra*.

These small molecules can be identified by any one or more of the screening assays discussed hereinabove and/or by any other screening techniques well known for those skilled in the art.

Uses of the herein disclosed molecules may also be based upon the positive functional assay hits disclosed and described below. Methods based upon those assay hits are also encompassed by the present invention.

## F. Anti-PRO Antibodies

The present invention further provides anti-PRO antibodies. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies.

### 1. Polyclonal Antibodies

The anti-PRO antibodies may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing

agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the PRO polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

## 2. Monoclonal Antibodies

The anti-PRO antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

The immunizing agent will typically include the PRO polypeptide or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103]. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against PRO. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such

as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, supra]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison et al., supra] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

*In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art.

### 3. Human and Humanized Antibodies

The anti-PRO antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')<sub>2</sub> or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin.



Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985) and Boerner et al., J. Immunol., 147(1):86-95 (1991)]. Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks *et al.*, Bio/Technology 10, 779-783 (1992); Lonberg *et al.*, Nature 368 856-859 (1994); Morrison, Nature 368, 812-13 (1994); Fishwild *et al.*, Nature Biotechnology 14, 845-51 (1996); Neuberger, Nature Biotechnology 14, 826 (1996); Lonberg and Huszar, Intern. Rev. Immunol. 13 65-93 (1995).

The antibodies may also be affinity matured using known selection and/or mutagenesis methods as described above. Preferred affinity matured antibodies have an affinity which is five times, more preferably

10 times, even more preferably 20 or 30 times greater than the starting antibody (generally murine, humanized or human) from which the matured antibody is prepared.

#### 4. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the PRO, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities [Milstein and Cuello, Nature, 305:537-539 (1983)]. Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker et al., EMBO J., 10:3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121:210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. F(ab')<sub>2</sub> bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al.*, Science 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')<sub>2</sub> fragments. These fragments are reduced in the presence of the

dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

5 Fab' fragments may be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby *et al.*, J. Exp. Med. 175:217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')<sub>2</sub> molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

10 Various technique for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, J. Immunol. 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V<sub>H</sub>) connected to a light-chain variable domain (V<sub>L</sub>) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V<sub>H</sub> and V<sub>L</sub> domains of one fragment are forced to pair with the complementary V<sub>L</sub> and V<sub>H</sub> domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber *et al.*, J. Immunol. 152:5368 (1994).  
20 Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al.*, J. Immunol. 147:60 (1991).

25 Exemplary bispecific antibodies may bind to two different epitopes on a given PRO polypeptide herein. Alternatively, an anti-PRO polypeptide arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular PRO polypeptide. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express a particular PRO polypeptide. These antibodies possess a PRO-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the PRO polypeptide and further binds tissue factor (TF).

## 35 5. Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate

antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [U.S. Patent No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

#### 6. Effector Function Engineering

It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, *e.g.*, the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) may be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, J. Exp Med., 176: 1191-1195 (1992) and Shopes, J. Immunol., 148: 2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al.* Cancer Research, 53: 2560-2565 (1993). Alternatively, an antibody can be engineered that has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al.*, Anti-Cancer Drug Design, 3: 219-230 (1989).

#### 7. Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, croton, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include <sup>212</sup>Bi, <sup>131</sup>I, <sup>131</sup>In, <sup>90</sup>Y, and <sup>186</sup>Re. Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.*, Science,

238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) that is conjugated to a cytotoxic agent (e.g., a radionucleotide).

#### 8. Immunoliposomes

The antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, Proc. Natl. Acad. Sci. USA, 82: 3688 (1985); Hwang *et al.*, Proc. Natl. Acad. Sci. USA, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al.*, J. Biol. Chem., 257: 286-288 (1982) via a disulfide-interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon *et al.*, J. National Cancer Inst., 81(19): 1484 (1989).

#### 9. Pharmaceutical Compositions of Antibodies

Antibodies specifically binding a PRO polypeptide identified herein, as well as other molecules identified by the screening assays disclosed hereinbefore, can be administered for the treatment of various disorders in the form of pharmaceutical compositions.

If the PRO polypeptide is intracellular and whole antibodies are used as inhibitors, internalizing antibodies are preferred. However, lipofections or liposomes can also be used to deliver the antibody, or an antibody fragment, into cells. Where antibody fragments are used, the smallest inhibitory fragment that specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable-region sequences of an antibody, peptide molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. See, e.g., Marasco *et al.*, Proc. Natl. Acad. Sci. USA, 90: 7889-7893 (1993). The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, *supra*.

5 The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include  
 10 polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and  $\gamma$  ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT<sup>TM</sup> (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release  
 15 of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide  
 20 interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

#### G. Uses for anti-PRO Antibodies

The anti-PRO antibodies of the invention have various utilities. For example, anti-PRO antibodies  
 25 may be used in diagnostic assays for PRO, *e.g.*, detecting its expression in specific cells, tissues, or serum. Various diagnostic assay techniques known in the art may be used, such as competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either heterogeneous or homogeneous phases [Zola, Monoclonal Antibodies: A Manual of Techniques, CRC Press, Inc. (1987) pp. 147-158]. The antibodies used in the diagnostic assays can be labeled with a detectable moiety. The detectable moiety should  
 30 be capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as <sup>3</sup>H, <sup>14</sup>C, <sup>32</sup>P, <sup>35</sup>S, or <sup>125</sup>I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase. Any method known in the art for conjugating the antibody to the detectable moiety may be employed, including those methods described by Hunter et al., Nature, **144**:945  
 35 (1962); David et al., Biochemistry, **13**:1014 (1974); Pain et al., J. Immunol. Meth., **40**:219 (1981); and Nygren, J. Histochem. and Cytochem., **30**:407 (1982).

Anti-PRO antibodies also are useful for the affinity purification of PRO from recombinant cell culture or natural sources. In this process, the antibodies against PRO are immobilized on a suitable support, such as a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody then is contacted with a sample containing the PRO to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the PRO, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent that will release the PRO from the antibody.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

### EXAMPLES

Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, Manassas, VA.

#### EXAMPLE 1: Extracellular Domain Homology Screening to Identify Novel Polypeptides and cDNA Encoding Therefor

The extracellular domain (ECD) sequences (including the secretion signal sequence, if any) from about 950 known secreted proteins from the Swiss-Prot public database were used to search EST databases. The EST databases included public databases (e.g., Dayhoff, GenBank), and proprietary databases (e.g. LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST-2 (Altschul et al., Methods in Enzymology 266:460-480 (1996)) as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequences. Those comparisons with a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, WA).

Using this extracellular domain homology screen, consensus DNA sequences were assembled relative to the other identified EST sequences using phrap. In addition, the consensus DNA sequences obtained were often (but not always) extended using repeated cycles of BLAST or BLAST-2 and phrap to extend the consensus sequence as far as possible using the sources of EST sequences discussed above.

Based upon the consensus sequences obtained as described above, oligonucleotides were then synthesized and used to identify by PCR a cDNA library that contained the sequence of interest and for use as probes to isolate a clone of the full-length coding sequence for a PRO polypeptide. Forward and reverse PCR primers generally range from 20 to 30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in length. In some cases, additional





were scraped off the plates and the DNA was isolated from the bacterial pellet using standard protocols, e.g. CsCl-gradient. The purified DNA was then carried on to the yeast protocols below.

The yeast methods were divided into three categories: (1) Transformation of yeast with the plasmid/cDNA combined vector; (2) Detection and isolation of yeast clones secreting amylase; and (3) PCR amplification of the insert directly from the yeast colony and purification of the DNA for sequencing and further analysis.

The yeast strain used was HD56-5A (ATCC-90785). This strain has the following genotype: MAT alpha, ura3-52, leu2-3, leu2-112, his3-11, his3-15, MAL<sup>+</sup>, SUC<sup>+</sup>, GAL<sup>+</sup>. Preferably, yeast mutants can be employed that have deficient post-translational pathways. Such mutants may have translocation deficient alleles in *sec71*, *sec72*, *sec62*, with truncated *sec71* being most preferred. Alternatively, antagonists (including antisense nucleotides and/or ligands) which interfere with the normal operation of these genes, other proteins implicated in this post translation pathway (e.g., SEC61p, SEC72p, SEC62p, SEC63p, TDJ1p or SSA1p-4p) or the complex formation of these proteins may also be preferably employed in combination with the amylase-expressing yeast.

Transformation was performed based on the protocol outlined by Gietz et al., Nucl. Acid. Res., 20:1425 (1992). Transformed cells were then inoculated from agar into YEPD complex media broth (100 ml) and grown overnight at 30°C. The YEPD broth was prepared as described in Kaiser et al., Methods in Yeast Genetics, Cold Spring Harbor Press, Cold Spring Harbor, NY, p. 207 (1994). The overnight culture was then diluted to about 2 x 10<sup>6</sup> cells/ml (approx. OD<sub>600</sub>=0.1) into fresh YEPD broth (500 ml) and regrown to 1 x 10<sup>7</sup> cells/ml (approx. OD<sub>600</sub>=0.4-0.5).

The cells were then harvested and prepared for transformation by transfer into GS3 rotor bottles in a Sorval GS3 rotor at 5,000 rpm for 5 minutes, the supernatant discarded, and then resuspended into sterile water, and centrifuged again in 50 ml falcon tubes at 3,500 rpm in a Beckman GS-6KR centrifuge. The supernatant was discarded and the cells were subsequently washed with LiAc/TE (10 ml, 10 mM Tris-HCl, 1 mM EDTA pH 7.5, 100 mM Li<sub>2</sub>OOCCH<sub>3</sub>), and resuspended into LiAc/TE (2.5 ml).

Transformation took place by mixing the prepared cells (100 µl) with freshly denatured single stranded salmon testes DNA (Lofstrand Labs, Gaithersburg, MD) and transforming DNA (1 µg, vol. < 10 µl) in microfuge tubes. The mixture was mixed briefly by vortexing, then 40% PEG/TE (600 µl, 40% polyethylene glycol-4000, 10 mM Tris-HCl, 1 mM EDTA, 100 mM Li<sub>2</sub>OOCCH<sub>3</sub>, pH 7.5) was added. This mixture was gently mixed and incubated at 30°C while agitating for 30 minutes. The cells were then heat shocked at 42°C for 15 minutes, and the reaction vessel centrifuged in a microfuge at 12,000 rpm for 5-10 seconds, decanted and resuspended into TE (500 µl, 10 mM Tris-HCl, 1 mM EDTA pH 7.5) followed by recentrifugation. The cells were then diluted into TE (1 ml) and aliquots (200 µl) were spread onto the selective media previously prepared in 150 mm growth plates (VWR).

Alternatively, instead of multiple small reactions, the transformation was performed using a single, large scale reaction, wherein reagent amounts were scaled up accordingly.

The selective media used was a synthetic complete dextrose agar lacking uracil (SCD-Ura) prepared as described in Kaiser et al., Methods in Yeast Genetics, Cold Spring Harbor Press, Cold Spring Harbor, NY,

p. 208-210 (1994). Transformants were grown at 30°C for 2-3 days.

The detection of colonies secreting amylase was performed by including red starch in the selective growth media. Starch was coupled to the red dye (Reactive Red-120, Sigma) as per the procedure described by Biely et al., *Anal. Biochem.*, 172:176-179 (1988). The coupled starch was incorporated into the SCD-Ura agar plates at a final concentration of 0.15% (w/v), and was buffered with potassium phosphate to a pH of 7.0 (50-100 mM final concentration).

The positive colonies were picked and streaked across fresh selective media (onto 150 mm plates) in order to obtain well isolated and identifiable single colonies. Well isolated single colonies positive for amylase secretion were detected by direct incorporation of red starch into buffered SCD-Ura agar. Positive colonies were determined by their ability to break down starch resulting in a clear halo around the positive colony visualized directly.

#### 4. Isolation of DNA by PCR Amplification

When a positive colony was isolated, a portion of it was picked by a toothpick and diluted into sterile water (30 µl) in a 96 well plate. At this time, the positive colonies were either frozen and stored for subsequent analysis or immediately amplified. An aliquot of cells (5 µl) was used as a template for the PCR reaction in a 25 µl volume containing: 0.5 µl KlenTaq (Clontech, Palo Alto, CA); 4.0 µl 10 mM dNTP's (Perkin Elmer-Cetus); 2.5 µl Kentaq buffer (Clontech); 0.25 µl forward oligo 1; 0.25 µl reverse oligo 2; 12.5 µl distilled water. The sequence of the forward oligonucleotide 1 was:

5'-TGTAACACGACGGCCAGTTAAATAGACCTGCAATTATTAATCT-3' (SEQ ID NO:1)

The sequence of reverse oligonucleotide 2 was:

5'-CAGGAAACAGCTATGACCACTGCACACCTGCAAATCCATT-3' (SEQ ID NO:2)

PCR was then performed as follows:

a.	Denature	92°C, 5 minutes
b.	3 cycles of:	
	Denature	92°C, 30 seconds
	Anneal	59°C, 30 seconds
	Extend	72°C, 60 seconds
c.	3 cycles of:	
	Denature	92°C, 30 seconds
	Anneal	57°C, 30 seconds
	Extend	72°C, 60 seconds
d.	25 cycles of:	
	Denature	92°C, 30 seconds
	Anneal	55°C, 30 seconds
	Extend	72°C, 60 seconds
e.	Hold	4°C

The underlined regions of the oligonucleotides annealed to the ADH promoter region and the amylase region, respectively, and amplified a 307 bp region from vector pSST-AMY.0 when no insert was present. Typically, the first 18 nucleotides of the 5' end of these oligonucleotides contained annealing sites for the sequencing primers. Thus, the total product of the PCR reaction from an empty vector was 343 bp. However, signal sequence-fused cDNA resulted in considerably longer nucleotide sequences.

Following the PCR, an aliquot of the reaction (5  $\mu$ l) was examined by agarose gel electrophoresis in a 1% agarose gel using a Tris-Borate-EDTA (TBE) buffering system as described by Sambrook et al., supra. Clones resulting in a single strong PCR product larger than 400 bp were further analyzed by DNA sequencing after purification with a 96 Qiaquick PCR clean-up column (Qiagen Inc., Chatsworth, CA).

### EXAMPLE 3: Isolation of cDNA Clones Using Signal Algorithm Analysis

Various polypeptide-encoding nucleic acid sequences were identified by applying a proprietary signal sequence finding algorithm developed by Genentech, Inc. (South San Francisco, CA) upon ESTs as well as clustered and assembled EST fragments from public (e.g., GenBank) and/or private (LIFESEQ®, Incyte Pharmaceuticals, Inc., Palo Alto, CA) databases. The signal sequence algorithm computes a secretion signal score based on the character of the DNA nucleotides surrounding the first and optionally the second methionine codon(s) (ATG) at the 5'-end of the sequence or sequence fragment under consideration. The nucleotides following the first ATG must code for at least 35 unambiguous amino acids without any stop codons. If the first ATG has the required amino acids, the second is not examined. If neither meets the requirement, the candidate sequence is not scored. In order to determine whether the EST sequence contains an authentic signal sequence, the DNA and corresponding amino acid sequences surrounding the ATG codon are scored using a set of seven sensors (evaluation parameters) known to be associated with secretion signals. Use of this algorithm resulted in the identification of numerous polypeptide-encoding nucleic acid sequences.

#### EXAMPLE 4: Isolation of cDNA clones Encoding Human PRO196

PRO196 was identified by screening the GenBank database using the computer program BLAST (Altshul et al., Methods in Enzymology 266:460-480 (1996)). The PRO196 sequence shows homology with known expressed sequence tag (EST) sequences T35448, T11442, and W77823. None of the known EST sequences have been identified as full length sequences, or described as ligands associated with the TIE receptors.

Following its identification, NL1 was cloned from a human fetal lung library prepared from mRNA purchased from Clontech, Inc. (Palo Alto, CA, USA), catalog # 6528-1, following the manufacturer's instructions. The library was screened by hybridization with synthetic oligonucleotide probes:

- (a) 5'-GCTGACGAACCAAGGCAACTACAAACTCCTGGT-3' (SEQ ID NO:5);  
(b) 5'-TGCGGCCGGACCAGTCCTCCATGGTCACCAGGAGTTTGTAG-3' (SEQ ID NO:6);  
(c) 5'-GGTGGTGAAGTCTGCTTGCCGTTGTGCCATGTAAA-3' (SEQ ID NO:7).

The nucleotide and amino acid sequences of PRO196 are shown in Figure 1 (SEQ ID NO:3) and Figure 2 (SEQ ID NO:4), respectively. PRO196 shows significant sequence identity with both the TIE1 and the TIE2 ligand.

A clone of PRO196 was deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, USA (ATCC) on September 18, 1997 under the terms of the Budapest Treaty, and has been assigned the deposit number 209280.

#### EXAMPLE 5: Isolation of cDNA clones Encoding Human PRO444

A cDNA sequence isolated in the amylase screen described in Example 2 above was designated DNA13121. Oligonucleotide probes were generated to this sequence and used to screen a human fetal lung library (LIB25) prepared as described in paragraph 1 of Example 2 above. The cloning vector was pRK5B (pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., *Science*, 253:1278-1280 (1991)), and the cDNA size cut was less than 2800 bp.

A full length clone was identified that contained a single open reading frame with an apparent translational initiation site at nucleotide positions 608-610 and ending at the stop codon found at nucleotide positions 959-961 (Figure 3, SEQ ID NO:8). The predicted polypeptide precursor is 117 amino acids long, has a calculated molecular weight of approximately 12,692 daltons and an estimated pI of approximately 7.50. Analysis of the full-length PRO444 sequence shown in Figure 4 (SEQ ID NO:9) evidences the presence of a signal peptide at amino acid 1 to about amino acid 16. An analysis of the Dayhoff database (version 35.45 SwissProt 35) evidenced homology between the PRO444 amino acid sequence and the following Dayhoff sequences: CEF44D12\_8, P\_R88452, YNE1\_CAEEL, A47312, AF009957\_1, and A06133\_1.

Clone DNA26846-1397 was deposited with the ATCC on October 27, 1998 and is assigned ATCC deposit no. 203406.

#### EXAMPLE 6: Isolation of cDNA clones Encoding Human PRO183, PRO185, PRO9940, PRO2630 and PRO6309

DNA molecules encoding the PRO183, PRO185, PRO9940, PRO2630 and PRO6309 polypeptides shown in the accompanying figures were obtained through GenBank.

#### EXAMPLE 7: Isolation of cDNA clones Encoding Human PRO210 and PRO217

A consensus DNA sequence was assembled using phrap as described in Example 1 above. In some cases, the consensus DNA sequence was extended using repeated cycles of blast and phrap to extend the consensus sequence as far as possible using the sources of EST sequences listed above. Based on this consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence. The library used to isolate DNA32279-1131 was fetal kidney.

cDNA clones were sequenced in their entirety. The entire nucleotide sequence of DNA32279-1131 is shown in Figure 9 (SEQ ID NO:14) and amino acid sequence of PRO210 is shown in Figure 10 (SEQ ID NO:15). The entire nucleotide sequence of DNA33094-1131 is shown in Figure 13 (SEQ ID NO:21) and amino acid sequence of PRO217 is shown in Figure 14 (SEQ ID NO:22).

#### EXAMPLE 8: Isolation of cDNA clones Encoding Human PRO215

A consensus DNA sequence was assembled relative to the other identified EST sequences as described in Example 1 above, wherein the consensus sequence was designated herein as DNA28748. Based on the DNA28748 consensus sequence, oligonucleotides were synthesized to identify by PCR a cDNA library that

contained the sequence of interest and for use as probes to isolate a clone of the full-length coding sequence for PRO215.

A pair of PCR primers (forward and reverse) were synthesized:

forward PCR primer 5'-GTGGCTGGCACACAATGAGATC-3' (SEQ ID NO:18)

reverse PCR primer 5'-CCAATGTGTGCAAGCGGTTGTG-3' (SEQ ID NO:19)

5 Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA28748 sequence which had the following nucleotide sequence:

hybridization probe

5'-TCAAGAGCCTGGACCTCAGCCACAATCTCATCTCTGACTTTGCCTGGAGC-3' (SEQ ID NO:20).

10 In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO215 gene using the probe oligonucleotide and one of the PCR primers.

15 RNA for construction of the cDNA libraries was isolated from human fetal lung tissue. The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., *Science*, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

20 DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO215 [herein designated as DNA32288-1132 and the derived protein sequence for PRO215.

25 The entire nucleotide sequence of DNA32288-1132 is shown in Figure 11 (SEQ ID NO:16). Clone DNA32288-1132 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 308-310 and ending at the stop codon at nucleotide positions 1591-1593 (Figure 11, the initiation and stop codons are circled). The predicted polypeptide precursor is 428 amino acids long (Figure 12). Clone DNA32288-1132 has been deposited with ATCC and is assigned ATCC deposit no. 209261.

Analysis of the amino acid sequence of the full-length PRO215 shows it has homology to member of the leucine rich repeat protein superfamily, including the leucine rich repeat protein and the SLIT protein.

#### EXAMPLE 9: Isolation of cDNA clones Encoding Human PRO242

30 An expressed sequence tag (EST) DNA database (LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA) was searched and an EST was identified which showed homology to a chemokine. Based on this sequence, oligonucleotides were synthesized to identify by PCR a cDNA library that contained the sequence of interest and for use as probes to isolate a clone of the full-length coding sequence for PRO242.

A pair of PCR primer (forward and reverse) were synthesized:

35 forward PCR primer 5'-GGATCAGGCAGGAGGAGTTTGGG-3' (SEQ ID NO:25)

reverse PCR primer 5'-GGATGGGTACAGACTTTCTTGCC-3' (SEQ ID NO:26)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA28709

sequence which had the following nucleotide sequence:

hybridization probe

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO242 gene using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal lung tissue. A cDNA clone was sequenced in entirety. The entire nucleotide sequence of DNA33785-1143 is shown in Figure 15 (SEQ ID NO:23). Clone DNA33785-1143 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 333-335 and ending at the stop codon at nucleotide positions 615-617 (Figure 16; SEQ ID NO:24). The predicted polypeptide precursor is 94 amino acids long (Figure 16).

Based on a BLAST and FastA sequence alignment analysis (using the ALIGN computer program) of the full-length sequence, PRO242 shows amino acid sequence identity to human macrophage inflammatory protein 1-alpha, rabbit macrophage inflammatory protein 1-beta, human LD78 and rabbit immune activation gene 2.

#### EXAMPLE 10: Isolation of cDNA clones Encoding Human PRO288

5'-CATAAAAGTTCCTGCACCATGACCAGAGACACAGTGTGTCAGTGTAAGA-3' (SEQ ID NO:30) was used to screen a human fetal lung cDNA library. To prepare the cDNA library, mRNA was isolated from human fetal lung tissue using reagents and protocols from Invitrogen, San Diego, CA (Fast Track 2). This RNA was used to generate an oligo dT primed cDNA library in the vector pRK5D using reagents and protocols from Life Technologies, Gaithersburg, MD (Super Script Plasmid System). In this procedure, the double stranded cDNA was sized to greater than 1000 bp and the SalI/NotI linked cDNA was cloned into XhoI/NotI cleaved vector. pRK5D is a cloning vector that has an sp6 transcription initiation site followed by an SfiI restriction enzyme site preceding the XhoI/NotI cDNA cloning sites.

A full length clone was identified (DNA35663-1129) that contained a single open reading frame with an apparent translational initiation site at nucleotide positions 157-159 and ending at the stop codon found at nucleotide positions 1315-1317 (Figure 17; SEQ ID NO:28). The clone is referred to as pRK5-35663 and is deposited as ATCC No. 209201.

The predicted polypeptide precursor is 386 amino acids long and has a calculated molecular weight of approximately 41.8 kDa. Sequence analysis indicated a N-terminal signal peptide (amino acids 1-55), followed by an ECD (amino acids 56-212), transmembrane-domain (amino acids 213-232) and intracellular region (amino acids 233-386). (Figure 18). The signal peptide cleavage site was confirmed by N-terminal protein sequencing of a PRO288 ECD immunoadhesin (not shown). This structure suggests that PRO288 is a type I transmembrane protein. PRO288 contains 3 potential N-linked glycosylation sites, at amino acid positions 127, 171 and 182. (Figure 18)

TNF receptor family proteins are typically characterized by the presence of multiple (usually four) cysteine-rich domains in their extracellular regions -- each cysteine-rich domain being approximately 45 amino acids long and containing approximately 6, regularly spaced, cysteine residues. Based on the crystal structure of the type 1 TNF receptor, the cysteines in each domain typically form three disulfide bonds in which usually cysteines 1 and 2, 3 and 5, and 4 and 6 are paired together. Like DR4, DR5, and DcR1, PRO288 contains two extracellular cysteine-rich pseudorepeats, whereas other identified mammalian TNFR family members contain three or more such domains [Smith et al., Cell, 76:959 (1994)].

Based on an alignment analysis of the PRO288 sequence shown in Figure 18 (SEQ ID NO:29), PRO288 shows more sequence identity to the ECD of DR4, DR5, or DcR1 than to other apoptosis-linked receptors, such as TNFR1, Fas/Apo-1 or DR3. The predicted intracellular sequence of PRO288 also shows more homology to the corresponding region of DR4 or DR5 as compared to TNFR1, Fas or DR3. The intracellular region of PRO288 is about 50 residues shorter than the intracellular regions identified for DR4 or DR5. It is presently believed that PRO288 may contain an truncated death domain (amino acids 340-364), which corresponds to the carboxy-terminal portion of the death domain sequences of DR4 and DR5. Five out of six amino acids that are essential for signaling by TNFR1 [Tartaglia et al., supra] and that are conserved or semi-conserved in DR4 and DR5, are absent in PRO288.

#### EXAMPLE 11: Isolation of cDNA clones Encoding Human PRO365

A consensus DNA sequence was assembled relative to other EST sequences using phrap as described in Example 1 above. This consensus sequence is herein designated DNA35613. Based on the DNA35613 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO365.

Forward and reverse PCR primers were synthesized:

forward PCR primer 5'-GGCTGGCCTGCAGAGATC-3' (SEQ ID NO:33)

forward PCR primer 5'-AATGTGACCACTGGACTCCC-3' (SEQ ID NO:34)

forward PCR primer 5'-AGGCTTGGAAGTCCCTTC-3' (SEQ ID NO:35)

reverse PCR primer 5'-AAGATTCTTGAGCGATTCCAGCTG-3' (SEQ ID NO:36)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA35613 sequence which had the following nucleotide sequence

hybridization probe

5'-AATCCCTGCTCTTCATGGTGACCTATGACGACGGAAGCACAAGACTG-3' (SEQ ID NO:37)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with one of the PCR primer pairs identified above. A positive library was then used to isolate clones encoding the PRO365 gene using the probe oligonucleotide and one of the PCR primers. RNA for construction of the cDNA libraries was isolated from human fetal kidney tissue.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO365 [herein designated as DNA46777-1253] (SEQ ID NO:31) and the derived protein sequence for

PRO365.

The entire nucleotide sequence of DNA46777-1253 is shown in Figure 19 (SEQ ID NO:31). Clone DNA46777-1253 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 15-17 and ending at the stop codon at nucleotide positions 720-722 (Figure 19). The predicted polypeptide precursor is 235 amino acids long (Figure 20). Important regions of the polypeptide sequence encoded by clone DNA46777-1253 have been identified and include the following: a signal peptide corresponding to amino acids 1-20 and multiple potential N-glycosylation sites. Clone DNA46777-1253 has been deposited with ATCC and is assigned ATCC deposit no. 209619.

Analysis of the amino acid sequence of the full-length PRO365 polypeptide suggests that portions of it possess significant homology to the human 2-19 protein, thereby indicating that PRO365 may be a novel human 2-19 protein homolog.

#### EXAMPLE 12: Isolation of cDNA clones Encoding Human PRO1361

Use of the signal sequence algorithm described in Example 3 above allowed identification of an EST cluster sequence from the Incyte database, designated Incyte cluster sequence 10685. This EST cluster sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (Lifeseq®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altschul et al., Methods in Enzymology 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). The consensus sequence obtained therefrom is herein designated DNA58839.

In light of an observed sequence homology between the DNA58839 sequence and an EST sequence contained within the Incyte EST clone no. 2967927, the Incyte EST clone no. 2967927 was purchased and the cDNA insert was obtained and sequenced. The sequence of this cDNA insert is shown in Figure 21 and is herein designated as DNA60783-1611.

Clone DNA60783-1611 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 142-144 and ending at the stop codon at nucleotide positions 1132-1134 (Figure 21). The predicted polypeptide precursor is 330 amino acids long (Figure 22). The full-length PRO1361 protein shown in Figure 22 has an estimated molecular weight of about 36,840 daltons and a pI of about 4.84. Analysis of the full-length PRO1361 sequence shown in Figure 22 (SEQ ID NO:39) evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 23, a transmembrane domain from about amino acid 266 to about amino acid 284, a leucine zipper pattern sequence from about amino acid 155 to about amino acid 176 and potential N-glycosylation sites from about amino acid 46 to about amino acid 49, from about amino acid 64 to about amino acid 67, from about amino acid 166 to about amino acid 169 and from about amino acid 191 to about amino acid 194. Clone DNA60783-1611 has been deposited with ATCC on August 18, 1998 and is assigned ATCC deposit no. 203130.



An analysis of the Dayhoff database (version 35.45 SwissProt 35), using a WU-BLAST2 sequence alignment analysis of the full-length sequence shown in Figure 22 (SEQ ID NO:39), evidenced significant homology between the PRO1361 amino acid sequence and the following Dayhoff sequences: I50620, G64876, PMCMSG102B\_2MSG104, HUMIGLVXY\_1 and PH1370.

5 **EXAMPLE 13: Isolation of cDNA clones Encoding Human PRO1308**

A consensus DNA sequence was assembled relative to other EST sequences using phrap as described in Example 1 above. The consensus sequence was extended then using repeated cycles of BLAST and phrap to extend the consensus sequence as far as possible using the sources of EST sequences discussed above. The extended consensus sequence is designated herein as "DNA35726". Based on the DNA35726 consensus  
10 sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO1308.

The following PCR primers (forward and reverse) were synthesized:

forward PCR primers 5'-TCCTGTGAGCACGTGGTGTG-3' (SEQ ID NO:42);

5'-GGGTGGGATAGACCTGCG-3' (SEQ ID NO:43);

15 5'-AAGGCCAAGAAGGCTGCC-3' (SEQ ID NO:44); and

5'-CCAGGCCTGCAGACCCAG-3' (SEQ ID NO:45).

reverse PCR primers 5'-CTTCCTCAGTCCTTCCAGGATATC-3' (SEQ ID NO:46);

5'-AAGCTGGATATCCTCCGTGTTGTC-3' (SEQ ID NO:47);

5'-CCTGAAGAGGCATGACTGCTTTTCTCA-3' (SEQ ID NO:48); and

20 5'-GGGGATAAACCTATTAATTATTGCTAC-3' (SEQ ID NO:49).

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA35726 sequence which had the following nucleotide sequence:

hybridization probe: 5'-AACGTCACCTACATCTCCTCGTGCCACATGCGCCAGGCCACCTG-3' (SEQ  
ID NO:50).

25 In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO1308 gene using the probe oligonucleotide and one of the PCR primers. RNA for construction of the cDNA libraries was isolated from a human SK-Lu-1 adenocarcinoma cell line.

30 DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO1308 (designated herein as DNA62306-1570 [Figure 23, SEQ ID NO:40]; and the derived protein sequence for PRO1308.

The entire coding sequence of PRO1308 is shown in Figure 23 (SEQ ID NO:40). Clone DNA62306-1570 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 17-19 and an apparent stop codon at nucleotide positions 806-808. The predicted polypeptide precursor is 263  
35 amino acids long. The full-length PRO1308 protein shown in Figure 24 has an estimated molecular weight of about 27,663 daltons and a pI of about 6.77. Additional features include a signal peptide at about amino acids 1-20, potential N-glycosylation sites at about amino acids 73-76 and 215-218, and regions of homology

with osteonectin domains at about amino acids 97-129 and 169-201.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using a WU-BLAST2 sequence alignment analysis of the full-length sequence shown in Figure 24 (SEQ ID NO:41), revealed significant homology between the PRO1308 amino acid sequence and Dayhoff sequence S55369. Homology was also revealed between the PRO1308 amino acid sequence and the following Dayhoff sequences: FSA\_HUMAN; P\_R20063, CELT13C2\_1, AGRI\_RAT, p\_W09406, G01639, SC1\_RAT, S60062, S51362, and IOV7\_CHICK.

Clone DNA62306-1570 has been deposited with ATCC and is assigned ATCC deposit no. 203254.

#### EXAMPLE 14: Isolation of cDNA clones Encoding Human PRO1183

Use of the signal sequence algorithm described in Example 3 above allowed identification of an EST cluster sequence from the Incyte database. This EST cluster sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altschul et al., Methods in Enzymology 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). The consensus sequence obtained therefrom is herein designated DNA56037.

In light of an observed sequence homology between the DNA56037 sequence and an EST sequence contained within the Incyte EST 1645856 (from a library constructed from prostate tumor tissue), the clone which includes EST 1645856 was purchased and the cDNA insert was obtained and sequenced. The sequence of this cDNA insert is shown in Figure 25 and is herein designated as DNA62880-1513.

The full length clone shown in Figure 25 contained a single open reading frame with an apparent translational initiation site at nucleotide positions 20-22 and ending at the stop codon found at nucleotide positions 1535-1537 (Figure 25; SEQ ID NO:51). The predicted polypeptide precursor (Figure 26, SEQ ID NO:52) is 505 amino acids long. The signal peptide is approximately at amino acids 1-23 of SEQ ID NO:52. PRO1183 has a calculated molecular weight of approximately 56,640 daltons and an estimated pI of approximately 6.1. Clone DNA62880-1513 was deposited with the ATCC on August 4, 1998 and is assigned ATCC deposit no. 203097.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using a WU-BLAST2 sequence alignment analysis of the full-length sequence shown in Figure 26 (SEQ ID NO:52), revealed sequence identity between the PRO1183 amino acid sequence and the following Dayhoff sequences: MTV010\_1, P\_W41604, S54021, AOFB\_HUMAN, NPAJ4683\_1, S74689, GEN13608, ACHC\_ACHFU, AB011173\_1 and PUO\_MICRU. It is believed that administration of PRO1183 or regulators thereof may treat certain oxidase disorders such as variegate porphyria.

#### EXAMPLE 15: Isolation of cDNA clones Encoding Human PRO1272

Use of the signal sequence algorithm described in Example 3 above allowed identification of an EST cluster sequence from the Incyte database. This EST cluster sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altshul et al., Methods in Enzymology 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). The consensus sequence obtained therefrom is herein designated DNA58753.

In light of an observed sequence homology between the DNA58753 sequence and an EST sequence contained within the EST clone 3049165, the Incyte clone (from a lung library) including EST 3049165 was purchased and the cDNA insert was obtained and sequenced. The sequence of this cDNA insert is shown in Figure 27 and is herein designated as DNA64896-1539.

The full length clone shown in Figure 27 contained a single open reading frame with an apparent translational initiation site at nucleotide positions 58-60 and ending at the stop codon found at nucleotide positions 556-558 (Figure 27; SEQ ID NO:53). The predicted polypeptide precursor (Figure 28, SEQ ID NO:54) is 166 amino acids long. The signal peptide is at about amino acids 1-23 of SEQ ID NO:54. PRO1272 has a calculated molecular weight of approximately 19,171 daltons and an estimated pI of approximately 8.26. Clone DNA64896-1539 was deposited with the ATCC on September 9, 1998 and is assigned ATCC deposit no. 203238.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using a WU-BLAST2 sequence alignment analysis of the full-length sequence shown in Figure 28 (SEQ ID NO:54), revealed sequence identity between the PRO1272 amino acid sequence and the following Dayhoff sequences (information from database incorporated herein): AF025474\_1, D69100, AE000757\_10, H69466, CELC50E3\_12, XLRANBP1\_1, YD67\_SCHPO, B69459, H36856, and FRU40755\_1.

#### EXAMPLE 16: Isolation of cDNA clones Encoding Human PRO1419

Use of the signal sequence algorithm described in Example 3 above allowed identification of an EST cluster sequence from the Incyte database. This EST cluster sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. One or more of the ESTs was derived from a diseased tonsil tissue library. The homology search was performed using the computer program BLAST or BLAST2 (Altshul et al., Methods in Enzymology 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). The consensus sequence obtained therefrom is herein designated DNA59761.

In light of an observed sequence homology between the DNA59761 sequence and an EST sequence contained within the Incyte EST 3815008, the clone including this EST was purchased and the cDNA insert was obtained and sequenced. The sequence of this cDNA insert is shown in Figure 29 and is herein designated as DNA71290-1630.

The full length clone shown in Figure 29 contained a single open reading frame with an apparent translational initiation site at nucleotide positions 86-88 and ending at the stop codon found at nucleotide positions 341-343 (Figure 29; SEQ ID NO:55). The predicted polypeptide precursor (Figure 30, SEQ ID NO:56) is 85 amino acids long with the signal peptide at about amino acids 1-17 of SEQ ID NO:56. PRO1419 has a calculated molecular weight of approximately 9,700 daltons and an estimated pI of approximately 9.55. Clone DNA71290-1630 was deposited with the ATCC on September 22, 1998 and is assigned ATCC deposit no. 203275.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using a WU-BLAST2 sequence alignment analysis of the full-length sequence shown in Figure 30 (SEQ ID NO:56), revealed sequence identity between the PRO1419 amino acid sequence and the following Dayhoff sequences (data incorporated herein): S07975 (B3-hordein), C48232, HOR7\_HORVU, GEN11764, S14970, AF020312\_1, STAJ3220\_1, CER07E3\_1, CEY37A1B\_4, and ATAC00423810.

#### EXAMPLE 17: Isolation of cDNA clones Encoding Human PRO4999

A consensus DNA sequence was assembled relative to other EST sequences using phrap as described in Example 1 above. This consensus sequence is herein designated DNA86634. Based on the DNA86634 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO4999.

PCR primers (forward and reverse) were synthesized:

forward PCR primer 5'-CCACTTGCCATGAACATGCCAC-3' (SEQ ID NO:59)

reverse PCR primer 5'-CCTCTTGACAGACATAGCGAGCCAC-3' (SEQ ID NO:60)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA86634 sequence which had the following nucleotide sequence

hybridization probe

5'-CACTCTTGTCTGTGGGAACCACACATCTTGCCACAACCTGTGGC-3' (SEQ ID NO:61)

RNA for construction of the cDNA libraries was isolated from human testis tissue. DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for a full-length PRO4999 polypeptide (designated herein as DNA96031-2664 [Figure 31, SEQ ID NO:57]) and the derived protein sequence for that PRO4999 polypeptide.

The full length clone identified above contained a single open reading frame with an apparent translational initiation site at nucleotide positions 42-44 and a stop signal at nucleotide positions 2283-2285 (Figure 31, SEQ ID NO:57). The predicted polypeptide precursor is 747 amino acids long, has a calculated molecular weight of approximately 82,710 daltons and an estimated pI of approximately 6.36. Analysis of the

full-length PRO4999 sequence shown in Figure 32 (SEQ ID NO:58) evidences the presence of a variety of important polypeptide domains as shown in Figure 32, wherein the locations given for those important polypeptide domains are approximate as described above. Clone DNA96031-2664 has been deposited with ATCC on June 15, 1999 and is assigned ATCC deposit no. 237-PTA.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using the ALIGN-2 sequence alignment analysis of the full-length sequence shown in Figure 32 (SEQ ID NO:58), evidenced sequence identity between the PRO4999 amino acid sequence and the following Dayhoff sequences: UROM\_HUMAN; FBNI\_HUMAN; GGU88872\_1; S52111; GEN12408; P\_R79478; P\_W48756; P\_R53087; P\_R14584; and S78549.

#### EXAMPLE 18: Isolation of cDNA clones Encoding Human PRO7170

Use of the signal sequence algorithm described in Example 3 above allowed identification of an EST cluster sequence from the LIFESEQ® database, Incyte Pharmaceuticals, Palo Alto, designated herein as CLU57836. This EST cluster sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., Genbank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altschul et al., Methods in Enzymology 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). The consensus sequence obtained therefrom is herein designated DNA58756.

In light of an observed sequence homology between the DNA58756 sequence and an EST sequence encompassed within clone no. 2251462 from the LIFESEQ® database, Incyte Pharmaceuticals, Palo Alto, CA, clone no. 2251462 was purchased and the cDNA insert was obtained and sequenced. It was found herein that that cDNA insert encoded a full-length protein. The sequence of this cDNA insert is shown in Figure 33 and is herein designated as DNA108722-2743.

Clone DNA108722-2743 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 60-62 and ending at the stop codon at nucleotide positions 1506-1508 (Figure 33). The predicted polypeptide precursor is 482 amino acids long (Figure 34). The full-length PRO7170 protein shown in Figure 34 has an estimated molecular weight of about 49,060 daltons and a pI of about 4.74. Analysis of the full-length PRO7170 sequence shown in Figure 34 (SEQ ID NO:63) evidences the presence of a variety of important polypeptide domains as shown in Figure 34, wherein the locations given for those important polypeptide domains are approximate as described above. Clone DNA108722-2743 has been deposited with ATCC on August 17, 1999 and is assigned ATCC Deposit No. 552-PTA.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using the ALIGN-2 sequence alignment analysis of the full-length sequence shown in Figure 34 (SEQ ID NO:63), evidenced sequence identity between the PRO7170 amino acid sequence and the following Dayhoff sequences: P\_Y12291, I47141, D88733\_1, DMC56G7\_1, P\_Y11606, HWP1\_CANAL, HSMUC5BEX\_1, HSU78550\_1, HSU70136\_1, and

SGS3\_DROME.

**EXAMPLE 19: Isolation of cDNA clones Encoding Human PRO248**

A consensus DNA sequence was assembled relative to the other identified EST sequences as described in Example 1 above, wherein the consensus sequence is designated herein as DNA33481. Based on the DNA33481 consensus sequence, oligonucleotides were synthesized to identify by PCR a cDNA library that contained the sequence of interest and for use as probes to isolate a clone of the full-length coding sequence for PRO248. Specifically, the following primers were used:

Forward primer 1 (SEQ ID NO:66): 5'-GTCTGACAGCCACTCCAGAG-3'

Hybridization probe (SEQ ID NO:67):

5'-TCTCCAATTTCTGGGCTTAGATAAGGCGCCTTCACCCCAGAAGTTCC-3'

Reverse primer 1 (SEQ ID NO:68): 5'-GTCCCAGGTTATAGTAAGAATTGG-3'

Forward primer 2 (SEQ ID NO:69): 5'-GTGTTGCGGTCAGTCCCATG-3'

Forward primer 3 (SEQ ID NO:70): 5'-GCTGTCTCCCATTTCCATGC-3'

Reverse primer 2 (SEQ ID NO:71): 5'-CGACTACCATGTCTTCATAATGTC-3'

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO248 gene using the probe oligonucleotide and one of the PCR primers. RNA for construction of the cDNA libraries was isolated from human fetal kidney tissue.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO248 [herein designated as DNA35674-1142] and the derived protein sequence for PRO248.

The entire nucleotide sequence of DNA35674-1142 is shown in Figure 35 (SEQ ID NO:64). Clone DNA35674-1142 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 66-68 and ending at the stop codon at nucleotide positions 1217-1219 (Figure 35; SEQ ID NO:64). The predicted polypeptide precursor is 364 amino acids long (Figure 36). Clone DNA35674-1142 has been deposited on October 28, 1997 with ATCC and is assigned ATCC deposit no. 209416.

Analysis of the amino acid sequence of the full-length PRO248 suggests that it has certain amino acid sequence identity with growth differentiation factor 3 from human and mouse.

**EXAMPLE 20: Isolation of cDNA clones Encoding Human PRO353**

A consensus DNA sequence was assembled relative to other EST sequences using phrap as described in Example 1 above. This consensus sequences is herein designated DNA36363. The consensus DNA sequence was extended using repeated cycles of BLAST and phrap to extend the consensus sequence as far as possible using the sources of EST sequences discussed above. Based on the DNA36363 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO353.

Based on the DNA36363 consensus sequence, forward and reverse PCR primers were synthesized as follows:

forward PCR primer 5'-TACAGGCCCCAGTCAGGACCAGGGG-3' (SEQ ID NO:74)

reverse PCR primer 5'-CTGAAGAAGTAGAGGCCGGGCACG-3' (SEQ ID NO:75).

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the DNA36363 consensus sequence which had the following nucleotide sequence:

hybridization probe

5 5'-CCCGGTGCTTGCGCTGCTGTGACCCCGGTACCTCCATGTACCCGG-3' (SEQ ID NO:76)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with one of the PCR primer pairs identified above. A positive library was then used to isolate clones encoding the PRO353 gene using the probe oligonucleotide and one of the PCR primers. RNA for construction of the cDNA libraries was isolated from human fetal kidney tissue.

10 DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO353 [herein designated as DNA41234-1242] (SEQ ID NO:72) and the derived protein sequence for PRO353.

The entire nucleotide sequence of DNA41234-1242 is shown in Figure 37 (SEQ ID NO:72). Clone DNA41234-1242 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 305-307 and ending at the stop codon at nucleotide positions 1148-1150 (Figure 37). The predicted polypeptide precursor is 281 amino acids long (Figure 38). Important regions of the amino acid sequence encoded by PRO353 include the signal peptide, corresponding to amino acids 1-26, the start of the mature protein at amino acid position 27, a potential N-glycosylation site, corresponding to amino acids 93-98 and a region which has homology to a 30 kd adipocyte complement-related protein precursor, corresponding to amino acids 99-281. Clone DNA41234-1242 has been deposited with the ATCC and is assigned ATCC deposit no. 209618.

Analysis of the amino acid sequence of the full-length PRO353 polypeptides suggests that portions of them possess significant homology to portions of human and murine complement proteins, thereby indicating that PRO353 may be a novel complement protein.

25

#### EXAMPLE 21: Isolation of cDNA clones Encoding Human PRO1318

The cDNA molecule corresponding to DNA73838-1674 as shown in Figure 39 (SEQ ID NO:77) was obtained from Curagen, Inc.

#### EXAMPLE 22: Isolation of cDNA clones Encoding Human PRO1600

A consensus DNA sequence was assembled relative to other EST sequences using phrap as described in Example 1 above. This consensus sequences is herein designated DNA75516. The consensus DNA sequence was extended using repeated cycles of BLAST and phrap to extend the consensus sequence as far as possible using the sources of EST sequences discussed above. Based on the DNA75516 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO1600.

35

Based on the DNA75516 consensus sequence, oligonucleotide probes were synthesized as follows:

5'-AGACATGGCTCAGTCACTGG-3' (SEQ ID NO:81)

5'-GACCCCTAAAGGGCCATAG-3' (SEQ ID NO:82).

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened with the probes identified above. RNA for construction of the cDNA libraries was isolated from human fetal heart tissue.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO1600 [herein designated as DNA77503-1686] (SEQ ID NO:79) and the derived protein sequence for PRO1600.

The entire nucleotide sequence of DNA77503-1686 is shown in Figure 41 (SEQ ID NO:79). Clone DNA77503-1686 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 6-8 and ending at the stop codon at nucleotide positions 408-410 (Figure 41). The predicted polypeptide precursor is 134 amino acids long (Figure 42). Important regions of the amino acid sequence of PRO1600 are shown in Figure 42. Clone DNA77503-1686 has been deposited with the ATCC and is assigned ATCC deposit no. 203362.

#### EXAMPLE 23: Isolation of cDNA clones Encoding Human PRO533

The EST sequence accession number AF007268, a murine fibroblast growth factor (FGF-15) was used to search various public EST databases (e.g., GenBank, Dayhoff, etc.). The search was performed using the computer program BLAST or BLAST2 [Altschul et al., *Methods in Enzymology*, 266:460-480 (1996)] as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequences. The search resulted in a hit with GenBank EST AA220994, which has been identified as stratagene NT2 neuronal precursor 937230.

Based on the Genbank EST AA220994 sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence. Forward and reverse PCR primers may range from 20 to 30 nucleotides (typically about 24), and are designed to give a PCR product of 100-1000 bp in length. The probe sequences are typically 40-55 bp (typically about 50) in length. In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification, as per *Ausubel et al., Current Protocols in Molecular Biology*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the PCR primers.

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified below. A positive library was then used to isolate clones encoding the PRO533 gene using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal retina. The cDNA libraries used to isolated the cDNA clones were constructed by standard methods using commercially available reagents (e.g., Invitrogen, San Diego, CA; Clontech, etc.) The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel



electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., *Science*, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

A cDNA clone was sequenced in its entirety. The full length nucleotide sequence of PRO533 is shown in Figure 45 (SEQ ID NO:85). Clone DNA49435-1219 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 459-461 (Figure 45; SEQ ID NO:85). The predicted polypeptide precursor is 216 amino acids long. Clone DNA47412-1219 has been deposited with ATCC and is assigned ATCC deposit no. ATCC 209480.

Based on a BLAST-2 and FastA sequence alignment analysis of the full-length sequence, PRO533 shows amino acid sequence identity to fibroblast growth factor (53%).

The oligonucleotide sequences used in the above procedure were the following:

FGF15.forward: 5'-ATCCGCCCAGATGGCTACAATGTGTA-3' (SEQ ID NO:87);

FGF15.probe: 5'-GCCTCCCGGTCTCCCTGAGCAGTGCCAAACAGCGGCAGTGTA-3' (SEQ ID NO:88);

FGF15.reverse: 5'-CCAGTCCGGTGACAAGCCCAAA-3' (SEQ ID NO:89).

#### EXAMPLE 24: Isolation of cDNA clones Encoding Human PRO301

A consensus DNA sequence designated herein as DNA35936 was assembled using phrap as described in Example 1 above. Based on this consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence.

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified below. A positive library was then used to isolate clones encoding the PRO301 gene using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal kidney.

A cDNA clone was sequenced in its entirety. The full length nucleotide sequence of native sequence PRO301 is shown in Figure 47 (SEQ ID NO:90). Clone DNA40628-1216 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 52-54 (Figure 47; SEQ ID NO:90). The predicted polypeptide precursor is 299 amino acids long with a predicted molecular weight of 32,583 daltons and pI of 8.29. Clone DNA40628-1216 has been deposited with ATCC and is assigned ATCC deposit No. ATCC 209432.

Based on a BLAST and FastA sequence alignment analysis of the full-length sequence, PRO301 shows amino acid sequence identity to A33 antigen precursor (30%) and coxsackie and adenovirus receptor protein (29%).

The oligonucleotide sequences used in the above procedure were the following:

OLI2162 (35936.f1) 5'-TCGCGGAGCTGTGTTCTGTTTCCC-3' (SEQ ID NO:92)

OLI2163 (35936.p1)

5'-TGATCGCGATGGGGACAAAGGCGCAAGCTCGAGAGGAACTGTTGTGCCT-3' (SEQ ID NO:93)

OLI2164 (35936.f2)

5'-ACACCTGGTTCAAAGATGGG-3' (SEQ ID NO:94)

OLI2165 (35936.r1)  
 5'-TAGGAAGAGTTGCTGAAGGCACGG-3' (SEQ ID NO:95)  
 OLI2166 (35936.f3)  
 5'-TTGCCTTACTCAGGTGCTAC-3' (SEQ ID NO:96)  
 OLI2167 (35936.r2)  
 5'-ACTCAGCAGTGGTAGGAAAG-3' (SEQ ID NO:97)

**EXAMPLE 25: Isolation of cDNA clones Encoding Human PRO187**

A proprietary expressed sequence tag (EST) DNA database (LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA) was searched and an EST (#843193) was identified which showed homology to fibroblast growth factor (FGF-8) also known as androgen-induced growth factor. mRNA was isolated from human fetal lung tissue using reagents and protocols from Invitrogen, San Diego, CA (Fast Track 2). The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents (e.g., Invitrogen, San Diego, CA, Life Technologies, Gaithersburg, MD). The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into the cloning vector pRK5D using reagents and protocols from Life Technologies, Gaithersburg, MD (Super Script Plasmid System). The double-stranded cDNA was sized to greater than 1000 bp and the SalI/NotI linkered cDNA was cloned into XhoI/NotI cleaved vector. pRK5D is a cloning vector that has an sp6 transcription initiation site followed by an SfiI restriction enzyme site preceding the XhoI/NotI cDNA cloning sites.

Several libraries from various tissue sources were screened by PCR amplification with the following oligonucleotide probes:

IN843193.f (OLI315) (SEQ ID NO:100)  
 5'-CAGTACGTGAGGGACCAGGGCGCCATGA-3'

IN843193.r (OLI 317) (SEQ ID NO:101)  
 5'-CCGGTGACCTGCACGTGCTTGCCA-3'

A positive library was then used to isolate clones encoding the PRO187 gene using one of the above oligonucleotides and the following oligonucleotide probe:

IN843193.p (OLI 316) (SEQ ID NO:102)  
 5'-GCGGATCTGCCGCCTGCTCANCTGGTCGGTCATGGCGCCCT-3'

A cDNA clone was sequenced in entirety. The entire nucleotide sequence of PRO187 (DNA27864-1155) is shown in Figure 49 (SEQ ID NO:98). Clone DNA27864-1155 contains a single open reading frame with an apparent translational initiation site at nucleotide position 1 (Figure 49; SEQ ID NO:98). The predicted polypeptide precursor is 205 amino acids long. Clone DNA27864-1155 has been deposited with the ATCC (designation: DNA27864-1155) and is assigned ATCC deposit no. ATCC 209375.

Based on a BLAST and FastA sequence alignment analysis (using the ALIGN computer program) of the full-length sequence, the PRO187 polypeptide shows 74% amino acid sequence identity (Blast score 310) to human fibroblast growth factor-8 (androgen-induced growth factor).

#### EXAMPLE 26: Isolation of cDNA clones Encoding Human PRO337

A cDNA sequence identified in the amylase screen described in Example 2 above is herein designated DNA42301. The DNA42301 sequence was then compared to other EST sequences using phrap as described in Example 1 above and a consensus sequence designated herein as DNA28761 was identified. Based on this consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence. In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO337 gene using the probe oligonucleotide and one of the PCR primers. RNA for construction of the cDNA libraries was isolated from human fetal brain.

A cDNA clone was sequenced in its entirety. The full length nucleotide sequence of DNA43316-1237 is shown in Figure 51 (SEQ ID NO:103). Clone DNA43316-1237 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 134-136 (Figure 51; SEQ ID NO:103). The predicted polypeptide precursor is 344 amino acids long. Clone DNA43316-1237 has been deposited with ATCC and is assigned ATCC deposit no. 209487

Based on a BLAST-2 and FastA sequence alignment analysis of the full-length sequence, PRO337 shows amino acid sequence identity to rat neurotrimin (97%).

#### EXAMPLE 27: Isolation of cDNA clones Encoding Human PRO1411

Use of the signal sequence algorithm described in Example 3 above allowed identification of an EST cluster sequence from an Incyte database. This EST cluster sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. One or more of the ESTs were derived from a thyroid tissue library. The homology search was performed using the computer program BLAST or BLAST2 (Altshul et al., Methods in Enzymology 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). The consensus sequence obtained therefrom is herein designated DNA56013.

In light of the sequence homology between the DNA56013 sequence and an EST sequence contained within the Incyte EST 1444225, the clone including this EST was purchased and the cDNA insert was obtained and sequenced. The sequence of this cDNA insert is shown in Figure 53 and is herein designated as DNA59212-1627.

The full length clone shown in Figure 53 contained a single open reading frame with an apparent translational initiation site at nucleotide positions 184-186 and ending at the stop codon found at nucleotide positions 1504-1506 (Figure 53; SEQ ID NO:105). The predicted polypeptide precursor (Figure 54, SEQ ID NO:106) is 440 amino acids long. The signal peptide is at about amino acids 1-21, and the cell attachment site is at about amino acids 301-303 of SEQ ID NO:106. PRO1411 has a calculated molecular weight of

approximately 42,208 daltons and an estimated pI of approximately 6.36. Clone DNA59212-1627 was deposited with the ATCC on September 9, 1998 and is assigned ATCC deposit no. 203245.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using a WU-BLAST2 sequence alignment analysis of the full-length sequence shown in Figure 54 (SEQ ID NO:106), revealed sequence identity between the PRO1411 amino acid sequence and the following Dayhoff sequences (data from database incorporated herein): MTV023\_19, P\_R05307, P\_W26348, P\_P82962, AF000949\_1, EBN1\_EBV, P\_R95107, GRP2\_PHAVU, P\_R81318, and S74439\_1.

#### EXAMPLE 28: Isolation of cDNA clones Encoding Human PRO4356

A consensus DNA sequence was assembled relative to other EST sequences using phrap as described in Example 1 above. This consensus sequence is designated herein "DNA80200". Based upon an observed homology between the DNA80200 consensus sequence and an EST sequence contained within Merck EST clone 248287, Merck EST clone 248287 was purchased and its insert obtained and sequenced, thereby providing DNA86576-2595.

The entire coding sequence of PRO4356 is shown in Figure 55 (SEQ ID NO:107). Clone DNA86576-2595 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 55-57, and an apparent stop codon at nucleotide positions 808-810. The predicted polypeptide precursor is 251 amino acids long. Clone DNA86576-2595 has been deposited with ATCC and is assigned ATCC deposit no. 203868. The full-length PRO4356 protein shown in Figure 56 has an estimated molecular weight of about 26,935 daltons and a pI of about 7.42.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using a WU-BLAST2 sequence alignment analysis of the full-length sequence shown in Figure 56 (SEQ ID NO:108), revealed homology between the PRO4356 amino acid sequence and the following Dayhoff sequences incorporated herein: RNMAPIAN\_1, UPAR\_BOVIN, S42152, AF007789\_1, UPAR\_RAT, UPAR\_MOUSE, P\_W31165, P\_W31168, P\_R44423 and P\_W26359.

#### EXAMPLE 29: Isolation of cDNA clones Encoding Human PRO246

A consensus DNA sequence was assembled relative to other EST sequences using phrap as described in Example 1 above. This consensus sequence is herein designated DNA30955. Based on the DNA30955 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO246.

A pair of PCR primers (forward and reverse) were synthesized:

forward PCR primer - 5'-AGGGTCTCCAGGAGAAAGACTC-3' (SEQ ID NO:111)

reverse PCR primer 5'-ATTGTGGGCCTTGCAGACATAGAC-3' (SEQ ID NO:112)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA30955 sequence which had the following nucleotide sequence

hybridization probe

5'-GGCCACAGCATCAAAACCTTAGAACTCAATGTACTGGTTCCTCCAGCTCC-3' (SEQ ID NO:113)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO246 gene using the probe oligonucleotide and one of the PCR primers.

5 RNA for construction of the cDNA libraries was isolated from human fetal liver tissue. DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO246 [herein designated as DNA35639-1172] (SEQ ID NO:109) and the derived protein sequence for PRO246.

10 The entire nucleotide sequence of DNA35639-1172 is shown in Figure 57 (SEQ ID NO:109). Clone DNA35639-1172 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 126-128 and ending at the stop codon at nucleotide positions 1296-1298 (Figure 57). The predicted polypeptide precursor is 390 amino acids long (Figure 58). Clone DNA35639-1172 has been deposited with ATCC and is assigned ATCC deposit no. ATCC 209396.

15 Analysis of the amino acid sequence of the full-length PRO246 polypeptide suggests that it possess significant homology to the human cell surface protein HCAR, thereby indicating that PRO246 may be a novel cell surface virus receptor.

EXAMPLE 30: Isolation of cDNA clones Encoding Human PRO265

20 A consensus DNA sequence was assembled relative to other EST sequences as described in Example 1 above using phrap. This consensus sequence is herein designated DNA33679. Based on the DNA33679 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO265.

PCR primers (two forward and one reverse) were synthesized:

forward PCR primer A: 5'-CGGTCTACCTGTATGGCAACC-3' (SEQ ID NO:116);

25 forward PCR primer B: 5'-GCAGGACAACCAGATAAACCAC-3' (SEQ ID NO:117);

reverse PCR primer 5'-ACGCAGATTTGAGAAGGCTGTC-3' (SEQ ID NO:118)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA33679 sequence which had the following nucleotide sequence

hybridization probe

30 5'-TTCACGGGCTGCTCTTGCCAGCTCTTGAAGCTTGAAGAGCTGCAC-3' (SEQ ID NO:119)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with PCR primer pairs identified above. A positive library was then used to isolate clones encoding the PRO265 gene using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal brain library.

35 DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO265 [herein designated as DNA36350-1158] (SEQ ID NO:114) and the derived protein sequence for PRO265.

The entire nucleotide sequence of DNA36350-1158 is shown in Figure 59 (SEQ ID NO:114). Clone DNA36350-1158 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 352-354 and ending at the stop codon at positions 2332-2334 (Figure 59). The predicted polypeptide precursor is 660 amino acids long (Figure 60). Clone DNA36350-1158 has been deposited with ATCC and is assigned ATCC deposit no. ATCC 209378.

- 5            Analysis of the amino acid sequence of the full-length PRO265 polypeptide suggests that portions of it possess significant homology to the fibromodulin and the fibromodulin precursor, thereby indicating that PRO265 may be a novel member of the leucine rich repeat family, particularly related to fibromodulin.

EXAMPLE 31: Isolation of cDNA clones Encoding Human PRO941

- 10            A consensus sequence was obtained relative to a variety of EST sequences as described in Example 1 above, wherein the consensus sequence obtained is herein designated DNA35941. Based on the DNA35941 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO941.

- 15            A pair of PCR primers (forward and reverse) were synthesized:

forward PCR primer 5'-CTTGACTGTCTCTGAATCTGCACCC-3' (SEQ ID NO:122)

reverse PCR primer 5'-AAGTGGTGGGAAGCCTCCAGTGTGG-3' (SEQ ID NO:123)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA35941 sequence which had the following nucleotide sequence

- 20            hybridization probe

5'-CCACTACGGTATTAGAGCAAAAGTTAAAAACCATCATGGTTCCTGGAGCAGC-3' (SEQ ID NO:124)

- 25            In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO941 gene using the probe oligonucleotide and one of the PCR primers. RNA for construction of the cDNA libraries was isolated from human fetal kidney tissue (LIB227).

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO941 [herein designated as DNA53906-1368] (SEQ ID NO:120) and the derived protein sequence for PRO941.

- 30            The entire nucleotide sequence of DNA53906-1368 is shown in Figure 61 (SEQ ID NO:120). Clone DNA53906-1368 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 37-39 and ending at the stop codon at nucleotide positions 2353-2355 (Figure 61). The predicted polypeptide precursor is 772 amino acids long (Figure 62). The full-length PRO941 protein shown in Figure 62 has an estimated molecular weight of about 87,002 daltons and a pI of about 4.64. Analysis of the full-length PRO941 sequence shown in Figure 62 (SEQ ID NO:121) evidences the presence of the following: a
- 35            signal peptide from about amino acid 1 to about amino acid 21, potential N-glycosylation sites from about amino acid 57 to about amino acid 60, from about amino acid 74 to about amino acid 77, from about amino

acid 419 to about amino acid 422, from about amino acid 437 to about amino acid 440, from about amino acid 508 to about amino acid 511, from about amino acid 515 to about amino acid 518, from about amino acid 516 to about amino acid 519 and from about amino acid 534 to about amino acid 537, and cadherin extracellular repeated domain signature sequences from about amino acid 136 to about amino acid 146 and from about amino acid 244 to about amino acid 254. Clone DNA53906-1368 has been deposited with ATCC on April 7, 1998 and is assigned ATCC deposit no. 209747.

Analysis of the amino acid sequence of the full-length PRO941 polypeptide suggests that it possesses significant sequence similarity to a cadherin protein, thereby indicating that PRO941 may be a novel cadherin protein family member. More specifically, an analysis of the Dayhoff database (version 35.45 SwissProt 35) evidenced significant homology between the PRO941 amino acid sequence and the following Dayhoff sequences, I50180, CADA\_CHICK, I50178, GEN12782, CADC\_HUMAN, P\_W25637, A38992, P\_R49731, D38992 and G02678.

#### EXAMPLE 32: Isolation of cDNA clones Encoding Human PRO10096

Use of the signal sequence algorithm described in Example 3 above allowed identification of an EST cluster sequence from the Incyte database, designated herein as 5086173H1. This EST cluster sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altschul et al., Methods in Enzymology 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). The consensus sequence obtained therefrom is herein designated DNA110880.

In light of an observed sequence homology between the DNA110880 sequence and an EST sequence encompassed within clone no. 5088384 from the Incyte database, clone no. 5088384 was purchased and the cDNA insert was obtained and sequenced. It was found herein that that cDNA insert encoded a full-length protein. The sequence of this cDNA insert is shown in Figure 63 and is herein designated as DNA125185-2506.

Clone DNA125185-2506 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 58-60 and ending at the stop codon at nucleotide positions 595-597 (Figure 63). The predicted polypeptide precursor is 179 amino acids long (Figure 64). The full-length PRO10096 protein shown in Figure 64 has an estimated molecular weight of about 20,011 daltons and a pI of about 8.10. Analysis of the full-length PRO10096 sequence shown in Figure 64 (SEQ ID NO:126) evidences the presence of a variety of important polypeptide domains as shown in Figure 64, wherein the locations given for those important polypeptide domains are approximate as described above. Clone DNA125185-2506 has been deposited with ATCC on December 7, 1999 and is assigned ATCC deposit no. 1031-PTA.

### EXAMPLE 33: Isolation of cDNA clones Encoding Human PRO6003

A cDNA clone (DNA83568-2692) encoding a native human PRO6003 polypeptide was identified using a yeast screen, in a human fetal kidney cDNA library that preferentially represents the 5' ends of the primary cDNA clones.

Clone DNA83568-2692 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 638-640 and ending at the stop codon at nucleotide positions 2225-2227 (Figure 65). The predicted polypeptide precursor is 529 amino acids long (Figure 66). The full-length PRO6003 protein shown in Figure 66 has an estimated molecular weight of about 59,583 daltons and a pI of about 6.36. Analysis of the full-length PRO6003 sequence shown in Figure 66 (SEQ ID NO:128) evidences the presence of a variety of important polypeptide domains as shown in Figure 66, wherein the locations given for those important polypeptide domains are approximate as described above. Clone DNA83568-2692 has been deposited with ATCC on July 20, 1999 and is assigned ATCC Deposit No. 386-PTA.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using the ALIGN-2 sequence alignment analysis of the full-length sequence shown in Figure 66 (SEQ ID NO:128), evidenced sequence identity between the PRO6003 amino acid sequence and the following Dayhoff sequences: P\_W58986, PTND7\_1, YKZ3\_YEAST, CEK04B12\_1, AB014464\_1, PCU07059\_1, S31213, CELF25E2\_2, AF036408\_1, and AB007932\_1.

### EXAMPLE 34: Isolation of cDNA clones Encoding Human PRO6004

A consensus sequence was obtained relative to a variety of EST sequences as described in Example 1 above, wherein the consensus sequence obtained is herein designated DNA85042. Based upon an observed homology between the DNA85042 consensus sequence and an EST sequence contained within Incyte EST clone no. 3078492, that clone was purchased and its insert obtained and sequenced. The sequence of that insert is herein designated as DNA92259 and is shown in Figures 67A-B (SEQ ID NO:129).

Clone DNA92259 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 16-18 and ending at the stop codon at nucleotide positions 1078-1080 (Figures 67A-B). The predicted polypeptide precursor is 354 amino acids long (Figure 68). The full-length PRO6004 protein shown in Figure 68 has an estimated molecular weight of about 38,719 daltons and a pI of about 6.12. Analysis of the full-length PRO6004 sequence shown in Figure 68 (SEQ ID NO:130) evidences the presence of a variety of important polypeptide domains as shown in Figure 68, wherein the locations given for those important polypeptide domains are approximate as described above.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using the ALIGN-2 sequence alignment analysis of the full-length sequence shown in Figure 68 (SEQ ID NO:130), evidenced sequence identity between the PRO6004 amino acid sequence and the following Dayhoff sequences: P\_W05152, LAMP\_HUMAN, P\_W05157, P\_W05155, I56551, OPCM\_RAT, AMAL\_DROME, DMU78177\_1, I37246 and NCA1\_HUMAN.



**EXAMPLE 35: Isolation of cDNA clones Encoding Human PRO350**

A consensus sequence was obtained relative to a variety of EST sequences as described in Example 1 above, wherein the consensus sequence obtained is herein designated DNA39493. Based on the DNA39493 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO350.

A pair of PCR primers (forward and reverse) were synthesized:

forward PCR primer 5'-TCAGGGCTGCCAGGAAGGAAGAGC-3' (SEQ ID NO:133)

reverse PCR primer 5'-GCAGGAGGAGAAGGTCTTCCAGAAGAAG-3' (SEQ ID NO:134)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA39493 sequence which had the following nucleotide sequence

hybridization probe

5'-AGAAGTTCAGTCAGCCCACAAGATGCCATTGTCCCCGGCCTCC-3' (SEQ ID NO:135)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO350 gene using the probe oligonucleotide and one of the PCR primers. RNA for construction of the cDNA libraries was isolated from human fetal kidney tissue.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO350 [herein designated as DNA44175-1314] (SEQ ID NO:131) and the derived protein sequence for PRO350.

The entire nucleotide sequence of DNA44175-1314 is shown in Figure 69 (SEQ ID NO:131). Clone DNA44175-1314 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 356-358 and ending at the stop codon at nucleotide positions 821-823 (Figure 69). The predicted polypeptide precursor is 155 amino acids long (Figure 70). The full-length PRO350 protein shown in Figure 70 has an estimated molecular weight of about 17,194 daltons and a pI of about 10.44. Analysis of the full-length PRO350 sequence shown in Figure 70 (SEQ ID NO:132) evidences the presence of a variety of important polypeptide domains as shown in Figure 70.

**EXAMPLE 36: Use of PRO as a hybridization probe**

The following method describes use of a nucleotide sequence encoding PRO as a hybridization probe.

DNA comprising the coding sequence of full-length or mature PRO as disclosed herein is employed as a probe to screen for homologous DNAs (such as those encoding naturally-occurring variants of PRO) in human tissue cDNA libraries or human tissue genomic libraries.

Hybridization and washing of filters containing either library DNAs is performed under the following high stringency conditions. Hybridization of radiolabeled PRO-derived probe to the filters is performed in a solution of 50% formamide, 5x SSC, 0.1% SDS, 0.1% sodium pyrophosphate, 50 mM sodium phosphate, pH 6.8, 2x Denhardt's solution, and 10% dextran sulfate at 42°C for 20 hours. Washing of the filters is performed in an aqueous solution of 0.1x SSC and 0.1% SDS at 42°C.

DNAs having a desired sequence identity with the DNA encoding full-length native sequence PRO can then be identified using standard techniques known in the art.

**EXAMPLE 37: Expression of PRO in *E. coli***

This example illustrates preparation of an unglycosylated form of PRO by recombinant expression in *E. coli*.

The DNA sequence encoding PRO is initially amplified using selected PCR primers. The primers should contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is pBR322 (derived from *E. coli*; see Bolivar et al., Gene, 2:95 (1977)) which contains genes for ampicillin and tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR amplified sequences are then ligated into the vector. The vector will preferably include sequences which encode for an antibiotic resistance gene, a trp promoter, a polyhis leader (including the first six STII codons, polyhis sequence, and enterokinase cleavage site), the PRO coding region, lambda transcriptional terminator, and an argU gene.

The ligation mixture is then used to transform a selected *E. coli* strain using the methods described in Sambrook et al., supra. Transformants are identified by their ability to grow on LB plates and antibiotic resistant colonies are then selected. Plasmid DNA can be isolated and confirmed by restriction analysis and DNA sequencing.

Selected clones can be grown overnight in liquid culture medium such as LB broth supplemented with antibiotics. The overnight culture may subsequently be used to inoculate a larger scale culture. The cells are then grown to a desired optical density, during which the expression promoter is turned on.

After culturing the cells for several more hours, the cells can be harvested by centrifugation. The cell pellet obtained by the centrifugation can be solubilized using various agents known in the art, and the solubilized PRO protein can then be purified using a metal chelating column under conditions that allow tight binding of the protein.

PRO may be expressed in *E. coli* in a poly-His tagged form, using the following procedure. The DNA encoding PRO is initially amplified using selected PCR primers. The primers will contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector, and other useful sequences providing for efficient and reliable translation initiation, rapid purification on a metal chelation column, and proteolytic removal with enterokinase. The PCR-amplified, poly-His tagged sequences are then ligated into an expression vector, which is used to transform an *E. coli* host based on strain 52 (W3110 fuhA(tonA) lon galE rpoHts(htpRts) clpP(lacIq). Transformants are first grown in LB containing 50 mg/ml carbenicillin at 30°C with shaking until an O.D.600 of 3-5 is reached. Cultures are then diluted 50-100 fold into CRAP media (prepared by mixing 3.57 g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.71 g sodium citrate•2H<sub>2</sub>O, 1.07 g KCl, 5.36 g Difco yeast extract, 5.36 g Sheffield hycase SF in 500 mL water, as well as 110 mM MPOS, pH 7.3, 0.55% (w/v) glucose and 7 mM MgSO<sub>4</sub>) and grown for approximately 20-30 hours at 30°C with shaking. Samples are removed to verify expression by SDS-PAGE analysis, and the bulk culture is centrifuged to pellet the cells.

Cell pellets are frozen until purification and refolding.

*E. coli* paste from 0.5 to 1 L fermentations (6-10 g pellets) is resuspended in 10 volumes (w/v) in 7 M guanidine, 20 mM Tris, pH 8 buffer. Solid sodium sulfite and sodium tetrathionate is added to make final concentrations of 0.1 M and 0.02 M, respectively, and the solution is stirred overnight at 4°C. This step results in a denatured protein with all cysteine residues blocked by sulfitolization. The solution is centrifuged at 40,000 rpm in a Beckman Ultracentrifuge for 30 min. The supernatant is diluted with 3-5 volumes of metal chelate column buffer (6 M guanidine, 20 mM Tris, pH 7.4) and filtered through 0.22 micron filters to clarify. The clarified extract is loaded onto a 5 ml Qiagen Ni-NTA metal chelate column equilibrated in the metal chelate column buffer. The column is washed with additional buffer containing 50 mM imidazole (Calbiochem, Utrol grade), pH 7.4. The protein is eluted with buffer containing 250 mM imidazole. Fractions containing the desired protein are pooled and stored at 4°C. Protein concentration is estimated by its absorbance at 280 nm using the calculated extinction coefficient based on its amino acid sequence.

The proteins are refolded by diluting the sample slowly into freshly prepared refolding buffer consisting of: 20 mM Tris, pH 8.6, 0.3 M NaCl, 2.5 M urea, 5 mM cysteine, 20 mM glycine and 1 mM EDTA. Refolding volumes are chosen so that the final protein concentration is between 50 to 100 micrograms/ml. The refolding solution is stirred gently at 4°C for 12-36 hours. The refolding reaction is quenched by the addition of TFA to a final concentration of 0.4% (pH of approximately 3). Before further purification of the protein, the solution is filtered through a 0.22 micron filter and acetonitrile is added to 2-10% final concentration. The refolded protein is chromatographed on a Poros R1/H reversed phase column using a mobile buffer of 0.1% TFA with elution with a gradient of acetonitrile from 10 to 80%. Aliquots of fractions with A280 absorbance are analyzed on SDS polyacrylamide gels and fractions containing homogeneous refolded protein are pooled. Generally, the properly refolded species of most proteins are eluted at the lowest concentrations of acetonitrile since those species are the most compact with their hydrophobic interiors shielded from interaction with the reversed phase resin. Aggregated species are usually eluted at higher acetonitrile concentrations. In addition to resolving misfolded forms of proteins from the desired form, the reversed phase step also removes endotoxin from the samples.

Fractions containing the desired folded PRO polypeptide are pooled and the acetonitrile removed using a gentle stream of nitrogen directed at the solution. Proteins are formulated into 20 mM Hepes, pH 6.8 with 0.14 M sodium chloride and 4% mannitol by dialysis or by gel filtration using G25 Superfine (Pharmacia) resins equilibrated in the formulation buffer and sterile filtered.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

#### EXAMPLE 38: Expression of PRO in mammalian cells

This example illustrates preparation of a potentially glycosylated form of PRO by recombinant expression in mammalian cells.

The vector, pRK5 (see EP 307,247, published March 15, 1989), is employed as the expression vector. Optionally, the PRO DNA is ligated into pRK5 with selected restriction enzymes to allow insertion of the PRO DNA using ligation methods such as described in Sambrook et al., supra. The resulting vector is called pRK5-

PRO.

In one embodiment, the selected host cells may be 293 cells. Human 293 cells (ATCC CCL 1573) are grown to confluence in tissue culture plates in medium such as DMEM supplemented with fetal calf serum and optionally, nutrient components and/or antibiotics. About 10  $\mu$ g pRK5-PRO DNA is mixed with about 1  $\mu$ g DNA encoding the VA RNA gene [Thimmappaya et al., *Cell*, 31:543 (1982)] and dissolved in 500  $\mu$ l of 1 mM Tris-HCl, 0.1 mM EDTA, 0.227 M  $\text{CaCl}_2$ . To this mixture is added, dropwise, 500  $\mu$ l of 50 mM HEPES (pH 7.35), 280 mM NaCl, 1.5 mM  $\text{NaPO}_4$ , and a precipitate is allowed to form for 10 minutes at 25°C. The precipitate is suspended and added to the 293 cells and allowed to settle for about four hours at 37°C. The culture medium is aspirated off and 2 ml of 20% glycerol in PBS is added for 30 seconds. The 293 cells are then washed with serum free medium, fresh medium is added and the cells are incubated for about 5 days.

Approximately 24 hours after the transfections, the culture medium is removed and replaced with culture medium (alone) or culture medium containing 200  $\mu$ Ci/ml  $^{35}\text{S}$ -cysteine and 200  $\mu$ Ci/ml  $^{35}\text{S}$ -methionine. After a 12 hour incubation, the conditioned medium is collected, concentrated on a spin filter, and loaded onto a 15% SDS gel. The processed gel may be dried and exposed to film for a selected period of time to reveal the presence of PRO polypeptide. The cultures containing transfected cells may undergo further incubation (in serum free medium) and the medium is tested in selected bioassays.

In an alternative technique, PRO may be introduced into 293 cells transiently using the dextran sulfate method described by Sompayrac et al., *Proc. Natl. Acad. Sci.*, 12:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700  $\mu$ g pRK5-PRO DNA is added. The cells are first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20% glycerol for 90 seconds, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5  $\mu$ g/ml bovine insulin and 0.1  $\mu$ g/ml bovine transferrin. After about four days, the conditioned media is centrifuged and filtered to remove cells and debris. The sample containing expressed PRO can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography.

In another embodiment, PRO can be expressed in CHO cells. The pRK5-PRO can be transfected into CHO cells using known reagents such as  $\text{CaPO}_4$  or DEAE-dextran. As described above, the cell cultures can be incubated, and the medium replaced with culture medium (alone) or medium containing a radiolabel such as  $^{35}\text{S}$ -methionine. After determining the presence of PRO polypeptide, the culture medium may be replaced with serum free medium. Preferably, the cultures are incubated for about 6 days, and then the conditioned medium is harvested. The medium containing the expressed PRO can then be concentrated and purified by any selected method.

Epitope-tagged PRO may also be expressed in host CHO cells. The PRO may be subcloned out of the pRK5 vector. The subclone insert can undergo PCR to fuse in frame with a selected epitope tag such as a poly-his tag into a Baculovirus expression vector. The poly-his tagged PRO insert can then be subcloned into a SV40 driven vector containing a selection marker such as DHFR for selection of stable clones. Finally, the CHO cells can be transfected (as described above) with the SV40 driven vector. Labeling may be

performed, as described above, to verify expression. The culture medium containing the expressed poly-His tagged PRO can then be concentrated and purified by any selected method, such as by Ni<sup>2+</sup>-chelate affinity chromatography.

PRO may also be expressed in CHO and/or COS cells by a transient expression procedure or in CHO cells by another stable expression procedure.

5 Stable expression in CHO cells is performed using the following procedure. The proteins are expressed as an IgG construct (immunoadhesin), in which the coding sequences for the soluble forms (e.g. extracellular domains) of the respective proteins are fused to an IgG1 constant region sequence containing the hinge, CH2 and CH2 domains and/or is a poly-His tagged form.

10 Following PCR amplification, the respective DNAs are subcloned in a CHO expression vector using standard techniques as described in Ausubel et al., Current Protocols of Molecular Biology, Unit 3.16, John Wiley and Sons (1997). CHO expression vectors are constructed to have compatible restriction sites 5' and 3' of the DNA of interest to allow the convenient shuttling of cDNA's. The vector used expression in CHO cells is as described in Lucas et al., Nucl. Acids Res. 24:9 (1774-1779 (1996), and uses the SV40 early promoter/enhancer to drive expression of the cDNA of interest and dihydrofolate reductase (DHFR). DHFR  
15 expression permits selection for stable maintenance of the plasmid following transfection.

Twelve micrograms of the desired plasmid DNA is introduced into approximately 10 million CHO cells using commercially available transfection reagents Superfect\* (Quiagen), Dosper\* or Fugene\* (Boehringer Mannheim). The cells are grown as described in Lucas et al., supra. Approximately 3 x 10<sup>7</sup> cells are frozen in an ampule for further growth and production as described below.

20 The ampules containing the plasmid DNA are thawed by placement into water bath and mixed by vortexing. The contents are pipetted into a centrifuge tube containing 10 mLs of media and centrifuged at 1000 rpm for 5 minutes. The supernatant is aspirated and the cells are resuspended in 10 mL of selective media (0.2 µm filtered PS20 with 5% 0.2 µm diafiltered fetal bovine serum). The cells are then aliquoted into a 100 mL spinner containing 90 mL of selective media. After 1-2 days, the cells are transferred into a 250 mL spinner filled with 150 mL selective growth medium and incubated at 37°C. After another 2-3 days, 250 mL, 500 mL and 2000 mL spinners are seeded with 3 x 10<sup>5</sup> cells/mL. The cell media is exchanged with fresh media by centrifugation and resuspension in production medium. Although any suitable CHO media may be employed, a production medium described in U.S. Patent No. 5,122,469, issued June 16, 1992 may actually be used. A 3L production spinner is seeded at 1.2 x 10<sup>6</sup> cells/mL. On day 0, the cell number pH is determined. On  
25 day 1, the spinner is sampled and sparging with filtered air is commenced. On day 2, the spinner is sampled, the temperature shifted to 33°C, and 30 mL of 500 g/L glucose and 0.6 mL of 10% antifoam (e.g., 35% polydimethylsiloxane emulsion, Dow Corning 365 Medical Grade Emulsion) taken. Throughout the production, the pH is adjusted as necessary to keep it at around 7.2. After 10 days, or until the viability dropped below 70%, the cell culture is harvested by centrifugation and filtering through a 0.22 µm filter. The  
30 filtrate was either stored at 4°C or immediately loaded onto columns for purification.

For the poly-His tagged constructs, the proteins are purified using a Ni-NTA column (Qiagen). Before purification, imidazole is added to the conditioned media to a concentration of 5 mM. The conditioned

media is pumped onto a 6 ml Ni-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min. at 4°C. After loading, the column is washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein is subsequently desalted into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.

Immunoadhesin (Fc-containing) constructs are purified from the conditioned media as follows. The conditioned medium is pumped onto a 5 ml Protein A column (Pharmacia) which had been equilibrated in 20 mM Na phosphate buffer, pH 6.8. After loading, the column is washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein is immediately neutralized by collecting 1 ml fractions into tubes containing 275  $\mu$ L of 1 M Tris buffer, pH 9. The highly purified protein is subsequently desalted into storage buffer as described above for the poly-His tagged proteins. The homogeneity is assessed by SDS polyacrylamide gels and by N-terminal amino acid sequencing by Edman degradation.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

#### EXAMPLE 39: Expression of PRO in Yeast

The following method describes recombinant expression of PRO in yeast.

First, yeast expression vectors are constructed for intracellular production or secretion of PRO from the ADH2/GAPDH promoter. DNA encoding PRO and the promoter is inserted into suitable restriction enzyme sites in the selected plasmid to direct intracellular expression of PRO. For secretion, DNA encoding PRO can be cloned into the selected plasmid, together with DNA encoding the ADH2/GAPDH promoter, a native PRO signal peptide or other mammalian signal peptide, or, for example, a yeast alpha-factor or invertase secretory signal/leader sequence, and linker sequences (if needed) for expression of PRO.

Yeast cells, such as yeast strain AB110, can then be transformed with the expression plasmids described above and cultured in selected fermentation media. The transformed yeast supernatants can be analyzed by precipitation with 10% trichloroacetic acid and separation by SDS-PAGE, followed by staining of the gels with Coomassie Blue stain.

Recombinant PRO can subsequently be isolated and purified by removing the yeast cells from the fermentation medium by centrifugation and then concentrating the medium using selected cartridge filters. The concentrate containing PRO may further be purified using selected column chromatography resins.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

#### EXAMPLE 40: Expression of PRO in Baculovirus-Infected Insect Cells

The following method describes recombinant expression of PRO in Baculovirus-infected insect cells.

The sequence coding for PRO is fused upstream of an epitope tag contained within a baculovirus expression vector. Such epitope tags include poly-his tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pVL1393 (Novagen). Briefly, the sequence encoding PRO or the desired portion of the coding sequence of PRO such as the sequence encoding the extracellular domain of a transmembrane protein or the sequence

encoding the mature protein if the protein is extracellular is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector.

Recombinant baculovirus is generated by co-transfecting the above plasmid and BaculoGold™ virus DNA (Pharmingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using lipofectin (commercially available from GIBCO-BRL). After 4 - 5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression are performed as described by O'Reilley et al., Baculovirus expression vectors: A Laboratory Manual, Oxford: Oxford University Press (1994).

Expressed poly-his tagged PRO can then be purified, for example, by Ni<sup>2+</sup>-chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert et al., Nature, 362:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM MgCl<sub>2</sub>; 0.1 mM EDTA; 10% glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10% glycerol, pH 7.8) and filtered through a 0.45 μm filter. A Ni<sup>2+</sup>-NTA agarose column (commercially available from Qiagen) is prepared with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is washed to baseline A<sub>280</sub> with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300 mM NaCl, 10% glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching A<sub>280</sub> baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. One mL fractions are collected and analyzed by SDS-PAGE and silver staining or Western blot with Ni<sup>2+</sup>-NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing the eluted His<sub>10</sub>-tagged PRO are pooled and dialyzed against loading buffer.

Alternatively, purification of the IgG tagged (or Fc tagged) PRO can be performed using known chromatography techniques, including for instance, Protein A or protein G column chromatography.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

#### EXAMPLE 41: Preparation of Antibodies that Bind PRO

This example illustrates preparation of monoclonal antibodies which can specifically bind PRO.

Techniques for producing the monoclonal antibodies are known in the art and are described, for instance, in Goding, supra. Immunogens that may be employed include purified PRO, fusion proteins containing PRO, and cells expressing recombinant PRO on the cell surface. Selection of the immunogen can be made by the skilled artisan without undue experimentation.

Mice, such as Balb/c, are immunized with the PRO immunogen emulsified in complete Freund's adjuvant and injected subcutaneously or intraperitoneally in an amount from 1-100 micrograms. Alternatively, the immunogen is emulsified in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) and injected into the animal's hind foot pads. The immunized mice are then boosted 10 to 12 days later with additional immunogen emulsified in the selected adjuvant. Thereafter, for several weeks, the mice may also

be boosted with additional immunization injections. Serum samples may be periodically obtained from the mice by retro-orbital bleeding for testing in ELISA assays to detect anti-PRO antibodies.

After a suitable antibody titer has been detected, the animals "positive" for antibodies can be injected with a final intravenous injection of PRO. Three to four days later, the mice are sacrificed and the spleen cells are harvested. The spleen cells are then fused (using 35 % polyethylene glycol) to a selected murine myeloma cell line such as P3X63AgU.1, available from ATCC, No. CRL 1597. The fusions generate hybridoma cells which can then be plated in 96 well tissue culture plates containing HAT (hypoxanthine, aminopterin, and thymidine) medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells will be screened in an ELISA for reactivity against PRO. Determination of "positive" hybridoma cells secreting the desired monoclonal antibodies against PRO is within the skill in the art.

The positive hybridoma cells can be injected intraperitoneally into syngeneic Balb/c mice to produce ascites containing the anti-PRO monoclonal antibodies. Alternatively, the hybridoma cells can be grown in tissue culture flasks or roller bottles. Purification of the monoclonal antibodies produced in the ascites can be accomplished using ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can be employed.

#### EXAMPLE 43: Purification of PRO Polypeptides Using Specific Antibodies

Native or recombinant PRO polypeptides may be purified by a variety of standard techniques in the art of protein purification. For example, pro-PRO polypeptide, mature PRO polypeptide, or pre-PRO polypeptide is purified by immunoaffinity chromatography using antibodies specific for the PRO polypeptide of interest. In general, an immunoaffinity column is constructed by covalently coupling the anti-PRO polypeptide antibody to an activated chromatographic resin.

Polyclonal immunoglobulins are prepared from immune sera either by precipitation with ammonium sulfate or by purification on immobilized Protein A (Pharmacia LKB Biotechnology, Piscataway, N.J.). Likewise, monoclonal antibodies are prepared from mouse ascites fluid by ammonium sulfate precipitation or chromatography on immobilized Protein A. Partially purified immunoglobulin is covalently attached to a chromatographic resin such as CnBr-activated SEPHAROSE™ (Pharmacia LKB Biotechnology). The antibody is coupled to the resin, the resin is blocked, and the derivative resin is washed according to the manufacturer's instructions.

Such an immunoaffinity column is utilized in the purification of PRO polypeptide by preparing a fraction from cells containing PRO polypeptide in a soluble form. This preparation is derived by solubilization of the whole cell or of a subcellular fraction obtained via differential centrifugation by the addition of detergent or by other methods well known in the art. Alternatively, soluble PRO polypeptide containing a signal sequence may be secreted in useful quantity into the medium in which the cells are grown.

A soluble PRO polypeptide-containing preparation is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of PRO polypeptide (*e.g.*, high ionic strength buffers in the presence of detergent). Then, the column is eluted under conditions that disrupt



**EXAMPLE 44: Drug Screening**

This invention is particularly useful for screening compounds by using PRO polypeptides or binding fragment thereof in any of a variety of drug screening techniques. The PRO polypeptide or fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the PRO polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, the formation of complexes between PRO polypeptide or a fragment and the agent being tested. Alternatively, one can examine the diminution in complex formation between the PRO polypeptide and its target cell or target receptors caused by the agent being tested.

Thus, the present invention provides methods of screening for drugs or any other agents which can affect a PRO polypeptide-associated disease or disorder. These methods comprise contacting such an agent with an PRO polypeptide or fragment thereof and assaying (i) for the presence of a complex between the agent and the PRO polypeptide or fragment, or (ii) for the presence of a complex between the PRO polypeptide or fragment and the cell, by methods well known in the art. In such competitive binding assays, the PRO polypeptide or fragment is typically labeled. After suitable incubation, free PRO polypeptide or fragment is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of the particular agent to bind to PRO polypeptide or to interfere with the PRO polypeptide/cell complex.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to a polypeptide and is described in detail in WO 84/03564, published on September 13, 1984. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. As applied to a PRO polypeptide, the peptide test compounds are reacted with PRO polypeptide and washed. Bound PRO polypeptide is detected by methods well known in the art. Purified PRO polypeptide can also be coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies can be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding PRO polypeptide specifically compete with a test compound for binding to PRO polypeptide or fragments thereof. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with PRO polypeptide.

**EXAMPLE 45: Rational Drug Design**

The goal of rational drug design is to produce structural analogs of biologically active polypeptide of interest (*i.e.*, a PRO polypeptide) or of small molecules with which they interact, *e.g.*, agonists, antagonists,

or inhibitors. Any of these examples can be used to fashion drugs which are more active or stable forms of the PRO polypeptide or which enhance or interfere with the function of the PRO polypeptide *in vivo* (c.f., Hodgson, Bio/Technology, 9: 19-21 (1991)).

In one approach, the three-dimensional structure of the PRO polypeptide, or of an PRO polypeptide-inhibitor complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the PRO polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of the PRO polypeptide may be gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous PRO polypeptide-like molecules or to identify efficient inhibitors. Useful examples of rational drug design may include molecules which have improved activity or stability as shown by Braxton and Wells, Biochemistry, 31:7796-7801 (1992) or which act as inhibitors, agonists, or antagonists of native peptides as shown by Athauda *et al.*, J. Biochem., 113:742-746 (1993).

It is also possible to isolate a target-specific antibody, selected by functional assay, as described above, and then to solve its crystal structure. This approach, in principle, yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced peptides. The isolated peptides would then act as the pharmacore.

By virtue of the present invention, sufficient amounts of the PRO polypeptide may be made available to perform such analytical studies as X-ray crystallography. In addition, knowledge of the PRO polypeptide amino acid sequence provided herein will provide guidance to those employing computer modeling techniques in place of or in addition to x-ray crystallography.

#### EXAMPLE 46: Mouse Kidney Mesangial Cell Proliferation Assay (Assay 92)

This assay shows that certain polypeptides of the invention act to induce proliferation of mammalian kidney mesangial cells and, therefore, are useful for treating kidney disorders associated with decreased mesangial cell function such as Berger disease or other nephropathies associated with Schönlein-Henoch purpura, celiac disease, dermatitis herpetiformis or Crohn disease. The assay is performed as follows. On day one, mouse kidney mesangial cells are plated on a 96 well plate in growth media (3:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium, 95% fetal bovine serum, 5% supplemented with 14 mM HEPES) and grown overnight. On day 2, PRO polypeptides are diluted at 2 concentrations (1% and 0.1%) in serum-free medium and added to the cells. Control samples are serum-free medium alone. On day 4, 20 $\mu$ l of the Cell Titer 96 Aqueous one solution reagent (Progenia) was added to each well and the colorimetric reaction was allowed to proceed for 2 hours. The absorbance (OD) is then measured at 490 nm. A positive in the assay is anything that gives an absorbance reading which is at least 15% above the control reading.

The following polypeptides tested positive in this assay: PRO1272.

**EXAMPLE 47: Detection of PRO Polypeptides That Affect Glucose or FFA Uptake by Primary Rat Adipocytes (Assay 94)**

This assay is designed to determine whether PRO polypeptides show the ability to affect glucose or FFA uptake by adipocyte cells. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of disorders where either the stimulation or inhibition of glucose uptake by adipocytes would be beneficial including, for example, obesity, diabetes or hyper- or hypo-insulinemia.

In a 96 well format, PRO polypeptides to be assayed are added to primary rat adipocytes, and allowed to incubate overnight. Samples are taken at 4 and 16 hours and assayed for glycerol, glucose and FFA uptake. After the 16 hour incubation, insulin is added to the media and allowed to incubate for 4 hours. At this time, a sample is taken and glycerol, glucose and FFA uptake is measured. Media containing insulin without the PRO polypeptide is used as a positive reference control. As the PRO polypeptide being tested may either stimulate or inhibit glucose and FFA uptake, results are scored as positive in the assay if greater than 1.5 times or less than 0.5 times the insulin control.

The following PRO polypeptides tested positive as either stimulators or inhibitors of glucose and/or FFA uptake in this assay: PRO196, PRO185, PRO210, PRO215, PRO242, PRO288, PRO1183, PRO1419, PRO9940, PRO301, PRO337 and PRO265.

**EXAMPLE 48: Stimulation of Adult Heart Hypertrophy (Assay 2)**

This assay is designed to measure the ability of various PRO polypeptides to stimulate hypertrophy of adult heart. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of various cardiac insufficiency disorders.

Ventricular myocytes freshly isolated from adult (250g) Sprague Dawley rats are plated at 2000 cell/well in 180  $\mu$ l volume. Cells are isolated and plated on day 1, the PRO polypeptide-containing test samples or growth medium only (negative control) (20  $\mu$ l volume) is added on day 2 and the cells are then fixed and stained on day 5. After staining, cell size is visualized wherein cells showing no growth enhancement as compared to control cells are given a value of 0.0, cells showing small to moderate growth enhancement as compared to control cells are given a value of 1.0 and cells showing large growth enhancement as compared to control cells are given a value of 2.0. Any degree of growth enhancement as compared to the negative control cells is considered positive for the assay.

The following PRO polypeptides tested positive in this assay: PRO301.

**EXAMPLE 49: Inhibition of Vascular Endothelial Growth Factor (VEGF) Stimulated Proliferation of Endothelial Cell Growth (Assay 9)**

The ability of various PRO polypeptides to inhibit VEGF stimulated proliferation of endothelial cells was tested. Polypeptides testing positive in this assay are useful for inhibiting endothelial cell growth in mammals where such an effect would be beneficial, e.g., for inhibiting tumor growth.

Specifically, bovine adrenal cortical capillary endothelial cells (ACE) (from primary culture, maximum of 12-14 passages) were plated in 96-well plates at 500 cells/well per 100 microliter. Assay media included low glucose DMEM, 10% calf serum, 2 mM glutamine, and 1X penicillin/streptomycin/fungizone. Control wells included the following: (1) no ACE cells added; (2) ACE cells alone; (3) ACE cells plus 5 ng/ml FGF; (4) ACE cells plus 3 ng/ml VEGF; (5) ACE cells plus 3 ng/ml VEGF plus 1 ng/ml TGF-beta; and (6) ACE cells plus 3 ng/ml VEGF plus 5 ng/ml LIF. The test samples, poly-his tagged PRO polypeptides (in 100 microliter volumes), were then added to the wells (at dilutions of 1%, 0.1% and 0.01%, respectively). The cell cultures were incubated for 6-7 days at 37°C/5% CO<sub>2</sub>. After the incubation, the media in the wells was aspirated, and the cells were washed 1X with PBS. An acid phosphatase reaction mixture (100 microliter; 0.1M sodium acetate, pH 5.5, 0.1% Triton X-100, 10 mM p-nitrophenyl phosphate) was then added to each well. After a 2 hour incubation at 37°C, the reaction was stopped by addition of 10 microliters 1N NaOH. Optical density (OD) was measured on a microplate reader at 405 nm.

The activity of PRO polypeptides was calculated as the percent inhibition of VEGF (3 ng/ml) stimulated proliferation (as determined by measuring acid phosphatase activity at OD 405 nm) relative to the cells without stimulation. TGF-beta was employed as an activity reference at 1 ng/ml, since TGF-beta blocks 70-90% of VEGF-stimulated ACE cell proliferation. The results are indicative of the utility of the PRO polypeptides in cancer therapy and specifically in inhibiting tumor angiogenesis. Numerical values (relative inhibition) are determined by calculating the percent inhibition of VEGF stimulated proliferation by the PRO polypeptides relative to cells without stimulation and then dividing that percentage into the percent inhibition obtained by TGF-β at 1 ng/ml which is known to block 70-90% of VEGF stimulated cell proliferation. The results are considered positive if the PRO polypeptide exhibits 30% or greater inhibition of VEGF stimulation of endothelial cell growth (relative inhibition 30% or greater).

The following polypeptide tested positive in this assay: PRO301, PRO187 and PRO246.

#### EXAMPLE 50: Stimulatory Activity in Mixed Lymphocyte Reaction (MLR) Assay (Assay 24)

This example shows that certain polypeptides of the invention are active as a stimulator of the proliferation of stimulated T-lymphocytes. Compounds which stimulate proliferation of lymphocytes are useful therapeutically where enhancement of an immune response is beneficial. A therapeutic agent may take the form of antagonists of the polypeptide of the invention, for example, murine-human chimeric, humanized or human antibodies against the polypeptide.

The basic protocol for this assay is described in Current Protocols in Immunology, unit 3.12; edited by J E Coligan, A M Kruisbeek, D H Marglies, E M Shevach, W Strober, National Institutes of Health, Published by John Wiley & Sons, Inc.

More specifically, in one assay variant, peripheral blood mononuclear cells (PBMC) are isolated from mammalian individuals, for example a human volunteer, by leukopheresis (one donor will supply stimulator PBMCs, the other donor will supply responder PBMCs). If desired, the cells are frozen in fetal bovine serum and DMSO after isolation. Frozen cells may be thawed overnight in assay media (37°C, 5% CO<sub>2</sub>) and then washed and resuspended to 3x10<sup>6</sup> cells/ml of assay media (RPMI; 10% fetal bovine serum, 1%

penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate). The stimulator PBMCs are prepared by irradiating the cells (about 3000 Rads).

The assay is prepared by plating in triplicate wells a mixture of:

100:1 of test sample diluted to 1% or to 0.1%,

50 :1 of irradiated stimulator cells, and

5 50 :1 of responder PBMC cells.

100 microliters of cell culture media or 100 microliter of CD4-IgG is used as the control. The wells are then incubated at 37°C, 5% CO<sub>2</sub> for 4 days. On day 5, each well is pulsed with tritiated thymidine (1.0 mCi/well; Amersham). After 6 hours the cells are washed 3 times and then the uptake of the label is evaluated.

In another variant of this assay, PBMCs are isolated from the spleens of Balb/c mice and C57B6 mice.

10 The cells are teased from freshly harvested spleens in assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate) and the PBMCs are isolated by overlaying these cells over Lympholyte M (Organon Teknika), centrifuging at 2000 rpm for 20 minutes, collecting and washing the mononuclear cell layer in assay media and resuspending the cells to 1x10<sup>7</sup> cells/ml of assay media. The assay is then conducted as described above.

15 Positive increases over control are considered positive with increases of greater than or equal to 180% being preferred. However, any value greater than control indicates a stimulatory effect for the test protein.

The following PRO polypeptides tested positive in this assay: PRO533 and PRO301.

#### EXAMPLE 51: PDB12 Cell Proliferation (Assay 29)

20 This example demonstrates that various PRO polypeptides have efficacy in inducing proliferation of PDB12 pancreatic ductal cells and are, therefore, useful in the therapeutic treatment of disorders which involve protein secretion by the pancreas, including diabetes, and the like.

25 PDB12 pancreatic ductal cells are plated on fibronectin coated 96 well plates at 1.5x10<sup>3</sup> cells per well in 100 µL/180 µL of growth media. 100 µL of growth media with the PRO polypeptide test sample or negative control lacking the PRO polypeptide is then added to well, for a final volume of 200 µL. Controls contain growth medium containing a protein shown to be inactive in this assay. Cells are incubated for 4 days at 37°C. 20 µL of Alamar Blue Dye (AB) is then added to each well and the fluorescent reading is measured at 4 hours post addition of AB, on a microtiter plate reader at 530 nm excitation and 590 nm emission. The standard employed is cells without Bovine Pituitary Extract (BPE) and with various concentrations of BPE. Buffer or 30 growth medium only controls from unknowns are run 2 times on each 96 well plate.

Percent increase in protein production is calculated by comparing the Alamar Blue Dye calculated protein concentration produced by the PRO polypeptide-treated cells with the Alamar Blue Dye calculated protein concentration produced by the negative control cells. A percent increase in protein production of greater than or equal to 25% as compared to the negative control cells is considered positive.

35 The following PRO polypeptides tested positive in this assay: PRO301.

**EXAMPLE 52: Guinea Pig Vascular Leak (Assay 32)**

This assay is designed to determine whether PRO polypeptides of the present invention show the ability to induce vascular permeability. Polypeptides testing positive in this assay are expected to be useful for the therapeutic treatment of conditions which would benefit from enhanced vascular permeability including, for example, conditions which may benefit from enhanced local immune system cell infiltration.

5 Hairless guinea pigs weighing 350 grams or more were anesthetized with Ketamine (75-80 mg/kg) and 5 mg/kg Xylazine intramuscularly. Test samples containing the PRO polypeptide or a physiological buffer without the test polypeptide are injected into skin on the back of the test animals with 100  $\mu$ l per injection site intradermally. There were approximately 16-24 injection sites per animal. One ml of Evans blue dye (1 % in PBS) is then injected intracardially. Skin vascular permeability responses to the compounds (*i.e.*, blemishes at the injection sites of injection) are visually scored by measuring the diameter (in mm) of blue-colored leaks from the site of injection at 1, 6 and 24 hours post administration of the test materials. The mm diameter of blueness at the site of injection is observed and recorded as well as the severity of the vascular leakage. Blemishes of at least 5 mm in diameter are considered positive for the assay when testing purified proteins, being indicative of the ability to induce vascular leakage or permeability. A response greater than 7 mm diameter is considered positive for conditioned media samples. Human VEGF at 0.1  $\mu$ g/100  $\mu$ l is used as a positive control, inducing a response of 4-8 mm diameter.

The following PRO polypeptides tested positive in this assay: PRO533.

**EXAMPLE 53: Retinal Neuron Survival (Assay 52)**

20 This example demonstrates that certain PRO polypeptides have efficacy in enhancing the survival of retinal neuron cells and, therefore, are useful for the therapeutic treatment of retinal disorders or injuries including, for example, treating sight loss in mammals due to retinitis pigmentosum, AMD, etc.

25 Sprague Dawley rat pups at postnatal day 7 (mixed population: glia and retinal neuronal types) are killed by decapitation following CO<sub>2</sub> anesthesia and the eyes are removed under sterile conditions. The neural retina is dissected away from the pigment epithelium and other ocular tissue and then dissociated into a single cell suspension using 0.25% trypsin in Ca<sup>2+</sup>, Mg<sup>2+</sup>-free PBS. The retinas are incubated at 37°C for 7-10 minutes after which the trypsin is inactivated by adding 1 ml soybean trypsin inhibitor. The cells are plated at 100,000 cells per well in 96 well plates in DMEM/F12 supplemented with N2 and with or without the specific test PRO polypeptide. Cells for all experiments are grown at 37°C in a water saturated atmosphere of 5% CO<sub>2</sub>. After 2-3 days in culture, cells are stained with calcein AM then fixed using 4% paraformaldehyde and stained with DAPI for determination of total cell count. The total cells (fluorescent) are quantified at 20X objective magnification using CCD camera and NIH image software for MacIntosh. Fields in the well are chosen at random.

35 The effect of various concentration of PRO polypeptides are reported herein where percent survival is calculated by dividing the total number of calcein AM positive cells at 2-3 days in culture by the total number of DAPI-labeled cells at 2-3 days in culture. Anything above 30% survival is considered positive.

The following PRO polypeptides tested positive in this assay using polypeptide concentrations within the range of 0.01% to 1.0% in the assay: PRO350.

**EXAMPLE 54: Proliferation of Rat Utricular Supporting Cells (Assay 54)**

This assay shows that certain polypeptides of the invention act as potent mitogens for inner ear supporting cells which are auditory hair cell progenitors and, therefore, are useful for inducing the regeneration of auditory hair cells and treating hearing loss in mammals. The assay is performed as follows. Rat UEC-4 utricular epithelial cells are aliquoted into 96 well plates with a density of 3000 cells/well in 200  $\mu$ l of serum-containing medium at 33°C. The cells are cultured overnight and are then switched to serum-free medium at 37°C. Various dilutions of PRO polypeptides (or nothing for a control) are then added to the cultures and the cells are incubated for 24 hours. After the 24 hour incubation,  $^3$ H-thymidine (1  $\mu$ Ci/well) is added and the cells are then cultured for an additional 24 hours. The cultures are then washed to remove unincorporated radiolabel, the cells harvested and Cpm per well determined. Cpm of at least 30% or greater in the PRO polypeptide treated cultures as compared to the control cultures is considered a positive in the assay.

The following polypeptides tested positive in this assay: PRO337.

**EXAMPLE 55: Rod Photoreceptor Cell Survival (Assay 56)**

This assay shows that certain polypeptides of the invention act to enhance the survival/proliferation of rod photoreceptor cells and, therefore, are useful for the therapeutic treatment of retinal disorders or injuries including, for example, treating sight loss in mammals due to retinitis pigmentosum, AMD, etc.

Sprague Dawley rat pups at 7 day postnatal (mixed population: glia and retinal neuronal cell types) are killed by decapitation following CO<sub>2</sub> anesthesia and the eyes are removed under sterile conditions. The neural retina is dissected away from the pigment epithelium and other ocular tissue and then dissociated into a single cell suspension using 0.25% trypsin in Ca<sup>2+</sup>, Mg<sup>2+</sup>-free PBS. The retinas are incubated at 37°C for 7-10 minutes after which the trypsin is inactivated by adding 1 ml soybean trypsin inhibitor. The cells are plated at 100,000 cells per well in 96 well plates in DMEM/F12 supplemented with N<sub>2</sub>. Cells for all experiments are grown at 37°C in a water saturated atmosphere of 5% CO<sub>2</sub>. After 2-3 days in culture, cells are fixed using 4% paraformaldehyde, and then stained using CellTracker Green CMFDA. Rho 4D2 (ascites or IgG 1:100), a monoclonal antibody directed towards the visual pigment rhodopsin is used to detect rod photoreceptor cells by indirect immunofluorescence. The results are calculated as % survival: total number of calcein - rhodopsin positive cells at 2-3 days in culture, divided by the total number of rhodopsin positive cells at time 2-3 days in culture. The total cells (fluorescent) are quantified at 20x objective magnification using a CCD camera and NIH image software for MacIntosh. Fields in the well are chosen at random.

The following polypeptides tested positive in this assay: PRO350.

**EXAMPLE 56: Skin Vascular Permeability Assay (Assay 64)**

This assay shows that certain polypeptides of the invention stimulate an immune response and induce inflammation by inducing mononuclear cell, eosinophil and PMN infiltration at the site of injection of the





**EXAMPLE 58: Induction of c-fos in Cortical Neurons (Assay 83)**

This assay is designed to determine whether PRO polypeptides show the ability to induce c-fos in cortical neurons. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of nervous system disorders and injuries where neuronal proliferation would be beneficial.

Cortical neurons are dissociated and plated in growth medium at 10,000 cells per well in 96 well plates. After approximately 2 cellular divisions, the cells are treated for 30 minutes with the PRO polypeptide or nothing (negative control). The cells are then fixed for 5 minutes with cold methanol and stained with an antibody directed against phosphorylated CREB. mRNA levels are then calculated using chemiluminescence. A positive in the assay is any factor that results in at least a 2-fold increase in c-fos message as compared to the negative controls.

The following PRO polypeptides tested positive in this assay: PRO288.

**EXAMPLE 59: Induction of Pancreatic  $\beta$ -Cell Precursor Differentiation (Assay 89)**

This assay shows that certain polypeptides of the invention act to induce differentiation of pancreatic  $\beta$ -cell precursor cells into mature pancreatic  $\beta$ -cells and, therefore, are useful for treating various insulin deficient states in mammals, including diabetes mellitus. The assay is performed as follows. The assay uses a primary culture of mouse fetal pancreatic cells and the primary readout is an alteration in the expression of markers that represent either  $\beta$ -cell precursors or mature  $\beta$ -cells. Marker expression is measured by real time quantitative PCR (RTQ-PCR); wherein the marker being evaluated is insulin.

The pancreata are dissected from E14 embryos (CD1 mice). The pancreata are then digested with collagenase/dispase in F12/DMEM at 37°C for 40 to 60 minutes (collagenase/dispase, 1.37 mg/ml, Boehringer Mannheim, #1097113). The digestion is then neutralized with an equal volume of 5% BSA and the cells are washed once with RPMI1640. At day 1, the cells are seeded into 12-well tissue culture plates (pre-coated with laminin, 20 $\mu$ g/ml in PBS, Boehringer Mannheim, #124317). Cells from pancreata from 1-2 embryos are distributed per well. The culture medium for this primary culture is 14F/1640. At day 2, the media is removed and the attached cells washed with RPMI/1640. Two mls of minimal media are added in addition to the protein to be tested. At day 4, the media is removed and RNA prepared from the cells and marker expression analyzed by real time quantitative RT-PCR. A protein is considered to be active in the assay if it increases the expression of the relevant  $\beta$ -cell marker as compared to untreated controls.

14F/1640 is RPMI1640 (Gibco) plus the following:

group A 1:1000

group B 1:1000

recombinant human insulin 10  $\mu$ g/ml

Aprotinin (50 $\mu$ g/ml) 1:2000 (Boehringer manheim #981532)

Bovine pituitary extract (BPE) 60 $\mu$ g/ml

Gentamycin 100 ng/ml

**Group A : (in 10ml PBS)**

Transferrin, 100mg (Sigma T2252)

**Epidermal Growth Factor, 100 $\mu$ g (BRL 100004)**

Triiodothyronine, 10  $\mu$ l of  $5 \times 10^{-6}$  M (Sigma T5516)

Ethanolamine, 100 $\mu$ l of 10<sup>-1</sup> M (Sigma E0135)

5 Phosphoethalamine, 100 $\mu$ l of 10<sup>-1</sup> M (Sigma P0503)

Selenium, 4 $\mu$ l of 10<sup>-1</sup> M (Aesar #12574)

Group C : (in 10ml 100% ethanol)

Hydrocortisone, 2 $\mu$ l of 5X10<sup>-3</sup> M (Sigma #H0135)

Progesterone, 100 $\mu$ l of 1X10<sup>-3</sup> M (Sigma #P6149)

10                      Forskolin, 500 $\mu$ l of 20mM (Calbiochem #344270)

**Minimal media:**

RPMI 1640 plus transferrin (10  $\mu\text{g/ml}$ ), insulin (1  $\mu\text{g/ml}$ ), gentamycin (100 ng/ml), aprotinin (50  $\mu\text{g/ml}$ ) and BPE (15  $\mu\text{g/ml}$ ).

**Defined media:**

15 RPMI 1640 plus transferrin (10  $\mu\text{g/ml}$ ), insulin (1  $\mu\text{g/ml}$ ), gentamycin (100 ng/ml) and aprotinin (50  $\mu\text{g/ml}$ ).

The following polypeptides were positive in this assay: PRO1361, PRO1308, PRO1600 and PRO4356.

20 **EXAMPLE 60: Pericyte c-Fos Induction (Assay 93)**

This assay shows that certain polypeptides of the invention act to induce the expression of c-fos in pericyte cells and, therefore, are useful not only as diagnostic markers for particular types of pericyte-associated tumors but also for giving rise to antagonists which would be expected to be useful for the therapeutic treatment of pericyte-associated tumors. Induction of c-fos expression in pericytes is also indicative of the induction of angiogenesis and, as such, PRO polypeptides capable of inducing the expression of c-fos would be expected to be useful for the treatment of conditions where induced angiogenesis would be beneficial including, for example, wound healing, and the like. Specifically, on day 1, pericytes are received from VEC Technologies and all but 5 ml of media is removed from flask. On day 2, the pericytes are trypsinized, washed, spun and then plated onto 96 well plates. On day 7, the media is removed and the pericytes are treated with 100  $\mu$ l of PRO polypeptide test samples and controls (positive control = DME+5% serum +/- PDGF at 500 ng/ml; negative control = protein 32). Replicates are averaged and SD/CV are determined. Fold increase over Protein 32 (buffer control) value indicated by chemiluminescence units (RLU) luminometer reading verses frequency is plotted on a histogram. Two-fold above Protein 32 value is considered positive for the assay. ASY Matrix: Growth media = low glucose DMEM = 20% FBS + 1X pen strep + 1X fungizone. Assay Media = low glucose DMEM +5% FBS.

The following polypeptides tested positive in this assay: PRO444 and PRO217.

**EXAMPLE 61: Detection of Polypeptides That Affect Glucose or FFA Uptake in Skeletal Muscle (Assay 106)**

This assay is designed to determine whether PRO polypeptides show the ability to affect glucose or FFA uptake by skeletal muscle cells. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of disorders where either the stimulation or inhibition of glucose uptake by skeletal muscle would be beneficial including, for example, diabetes or hyper- or hypo-insulinemia.

In a 96 well format, PRO polypeptides to be assayed are added to primary rat differentiated skeletal muscle, and allowed to incubate overnight. Then fresh media with the PRO polypeptide and +/- insulin are added to the wells. The sample media is then monitored to determine glucose and FFA uptake by the skeletal muscle cells. The insulin will stimulate glucose and FFA uptake by the skeletal muscle, and insulin in media without the PRO polypeptide is used as a positive control, and a limit for scoring. As the PRO polypeptide being tested may either stimulate or inhibit glucose and FFA uptake, results are scored as positive in the assay if greater than 1.5 times or less than 0.5 times the insulin control.

The following PRO polypeptides tested positive as either stimulators or inhibitors of glucose and/or FFA uptake in this assay: PRO196, PRO183, PRO185, PRO215, PRO288, PRO1361, PRO1600, PRO4999, PRO7170, PRO533 and PRO187.

**EXAMPLE 62: Fetal Hemoglobin Induction in an Erythroblastic Cell Line (Assay 107)**

This assay is useful for screening PRO polypeptides for the ability to induce the switch from adult hemoglobin to fetal hemoglobin in an erythroblastic cell line. Molecules testing positive in this assay are expected to be useful for therapeutically treating various mammalian hemoglobin-associated disorders such as the various thalassemias. The assay is performed as follows. Erythroblastic cells are plated in standard growth medium at 1000 cells/well in a 96 well format. PRO polypeptides are added to the growth medium at a concentration of 0.2% or 2% and the cells are incubated for 5 days at 37°C. As a positive control, cells are treated with 100µM hemin and as a negative control, the cells are untreated. After 5 days, cell lysates are prepared and analyzed for the expression of gamma globin (a fetal marker). A positive in the assay is a gamma globin level at least 2-fold above the negative control.

The following polypeptides tested positive in this assay: PRO1419.

**EXAMPLE 63: Chondrocyte Re-differentiation Assay (Assay 110)**

This assay shows that certain polypeptides of the invention act to induce redifferentiation of chondrocytes, therefore, are expected to be useful for the treatment of various bone and/or cartilage disorders such as, for example, sports injuries and arthritis. The assay is performed as follows. Porcine chondrocytes are isolated by overnight collagenase digestion of articular cartilage of metacarpophalangeal joints of 4-6 month old female pigs. The isolated cells are then seeded at 25,000 cells/cm<sup>2</sup> in Ham F-12 containing 10% FBS and 4 µg/ml gentamycin. The culture media is changed every third day and the cells are then seeded in 96 well plates at 5,000 cells/well in 100µl of the same media without serum and 100 µl of the test PRO polypeptide, 5 nM staurosporin (positive control) or medium alone (negative control) is added to give a final volume of 200 µl/well. After 5 days of incubation at 37°C, a picture of each well is taken and the

differentiation state of the chondrocytes is determined. A positive result in the assay occurs when the redifferentiation of the chondrocytes is determined to be more similar to the positive control than the negative control.

The following polypeptide tested positive in this assay: PRO215, PRO353, PRO365, PRO1272, PRO301 and PRO337.

5

**EXAMPLE 64: Chondrocyte Proliferation Assay (Assay 111)**

This assay is designed to determine whether PRO polypeptides of the present invention show the ability to induce the proliferation and/or redifferentiation of chondrocytes in culture. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of various bone and/or cartilage disorders such as, for example, sports injuries and arthritis.

10

Porcine chondrocytes are isolated by overnight collagenase digestion of articular cartilage of the metacarpophalangeal joint of 4-6 month old female pigs. The isolated cells are then seeded at 25,000 cells/cm<sup>2</sup> in Ham F-12 containing 10% FBS and 4 µg/ml gentamycin. The culture media is changed every third day and the cells are reseeded to 25,000 cells/cm<sup>2</sup> every five days. On day 12, the cells are seeded in 96 well plates at 5,000 cells/well in 100 µl of the same media without serum and 100 µl of either serum-free medium (negative control), staurosporin (final concentration of 5 nM; positive control) or the test PRO polypeptide are added to give a final volume of 200 µl/well. After 5 days at 37°C, 20 µl of Alamar blue is added to each well and the plates are incubated for an additional 3 hours at 37°C. The fluorescence is then measured in each well (Ex: 530 nm; Em: 590 nm). The fluorescence of a plate containing 200 µl of the serum-free medium is measured to obtain the background. A positive result in the assay is obtained when the fluorescence of the PRO polypeptide treated sample is more like that of the positive control than the negative control.

15

20

The following PRO polypeptides tested positive in this assay: PRO215, PRO217, PRO248, PRO1361, PRO1419, PRO533 and PRO265.

25

**EXAMPLE 65: Mouse Mesangial Cell Inhibition Assay (Assay 114)**

This assay is designed to determine whether PRO polypeptides of the present invention show the ability to inhibit the proliferation of mouse mesangial cells in culture. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of such diseases or conditions where inhibition of mesangial cell proliferation would be beneficial such as, for example, cystic renal dysplasia, polycystic kidney disease, or other kidney disease associated with abnormal mesangial cell proliferation, renal tumors, and the like.

30

On day 1, mouse mesangial cells are plated on a 96 well plate in growth medium (a 3:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium, 95%; fetal bovine serum, 5%; supplemented with 14mM HEPES) and then are allowed to grow overnight. On day 2, the PRO polypeptide is diluted at 2 different concentrations (1%, 0.1%) in serum-free medium and is added to the cells. The negative control is growth medium without added PRO polypeptide. After the cells are allowed to incubate for 48 hours, 20 µl of the Cell Titer 96 Aqueous one solution reagent (Promega) is added to each well and the colorimetric reaction

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is allowed to proceed for 2 hours. The absorbance (OD) is then measured at 490 nm. A positive in the assay is an absorbance reading which is at least 10% above the negative control.

The following PRO polypeptides tested positive in this assay: PRO1318.

**EXAMPLE 66: Induction of Pancreatic  $\beta$ -Cell Precursor Proliferation (Assay 117)**

This assay shows that certain polypeptides of the invention act to induce an increase in the number of pancreatic  $\beta$ -cell precursor cells and, therefore, are useful for treating various insulin deficient states in mammals, including diabetes mellitus. The assay is performed as follows. The assay uses a primary culture of mouse fetal pancreatic cells and the primary readout is an alteration in the expression of markers that represent either  $\beta$ -cell precursors or mature  $\beta$ -cells. Marker expression is measured by real time quantitative PCR (RTQ-PCR); wherein the marker being evaluated is a transcription factor called Pdx1.

The pancreata are dissected from E14 embryos (CD1 mice). The pancreata are then digested with collagenase/dispase in F12/DMEM at 37°C for 40 to 60 minutes (collagenase/dispase, 1.37 mg/ml, Boehringer Mannheim, #1097113). The digestion is then neutralized with an equal volume of 5% BSA and the cells are washed once with RPMI1640. At day 1, the cells are seeded into 12-well tissue culture plates (pre-coated with laminin, 20 $\mu$ g/ml in PBS, Boehringer Mannheim, #124317). Cells from pancreata from 1-2 embryos are distributed per well. The culture medium for this primary culture is 14F/1640. At day 2, the media is removed and the attached cells washed with RPMI/1640. Two mls of minimal media are added in addition to the protein to be tested. At day 4, the media is removed and RNA prepared from the cells and marker expression analyzed by real time quantitative RT-PCR. A protein is considered to be active in the assay if it increases the expression of the relevant  $\beta$ -cell marker as compared to untreated controls.

14F/1640 is RPMI1640 (Gibco) plus the following:

group A 1:1000

group B 1:1000

recombinant human insulin 10  $\mu$ g/ml

Aprotinin (50 $\mu$ g/ml) 1:2000 (Boehringer manheim #981532)

Bovine pituitary extract (BPE) 60 $\mu$ g/ml

Gentamycin 100 ng/ml

Group A : (in 10ml PBS)

Transferrin, 100mg (Sigma T2252)

Epidermal Growth Factor, 100 $\mu$ g (BRL 100004)

Triiodothyronine, 10 $\mu$ l of 5x10<sup>-6</sup> M (Sigma T5516)

Ethanolamine, 100 $\mu$ l of 10<sup>-1</sup> M (Sigma E0135)

Phosphoethalamine, 100 $\mu$ l of 10<sup>-1</sup> M (Sigma P0503)

Selenium, 4 $\mu$ l of 10<sup>-1</sup> M (Aesar #12574)

Group C : (in 10ml 100% ethanol)

Hydrocortisone, 2 $\mu$ l of 5X10<sup>-3</sup> M (Sigma #H0135)

Progesterone, 100 $\mu$ l of 1X10<sup>-3</sup> M (Sigma #P6149)

Forskolin, 500 $\mu$ l of 20mM (Calbiochem #344270)

Minimal media:

RPMI 1640 plus transferrin (10  $\mu$ g/ml), insulin (1  $\mu$ g/ml), gentamycin (100 ng/ml), aprotinin (50  $\mu$ g/ml) and BPE (15  $\mu$ g/ml).

Defined media:

5 RPMI 1640 plus transferrin (10  $\mu$ g/ml), insulin (1  $\mu$ g/ml), gentamycin (100 ng/ml) and aprotinin (50  $\mu$ g/ml).

The following polypeptides tested positive in this assay: PRO183, PRO185, PRO288.

EXAMPLE 67: *In Vitro* Antitumor Assay (Assay 161)

10 The antiproliferative activity of various PRO polypeptides was determined in the investigational, disease-oriented *in vitro* anti-cancer drug discovery assay of the National Cancer Institute (NCI), using a sulforhodamine B (SRB) dye binding assay essentially as described by Skehan et al., J. Natl. Cancer Inst. 82:1107-1112 (1990). The 60 tumor cell lines employed in this study ("the NCI panel"), as well as conditions for their maintenance and culture *in vitro* have been described by Monks et al., J. Natl. Cancer Inst. 83:757-15 766 (1991). The purpose of this screen is to initially evaluate the cytotoxic and/or cytostatic activity of the test compounds against different types of tumors (Monks et al., *supra*; Boyd, Cancer: Princ. Pract. Oncol. Update 3(10):1-12 [1989]).

Cells from approximately 60 human tumor cell lines were harvested with trypsin/EDTA (Gibco), washed once, resuspended in IMEM and their viability was determined. The cell suspensions were added by pipet (100  $\mu$ L volume) into separate 96-well microtiter plates. The cell density for the 6-day incubation was less than for the 2-day incubation to prevent overgrowth. Inoculates were allowed a preincubation period of 24 hours at 37°C for stabilization. Dilutions at twice the intended test concentration were added at time zero in 100  $\mu$ L aliquots to the microtiter plate wells (1:2 dilution). Test compounds were evaluated at five half-log dilutions (1000 to 100,000-fold). Incubations took place for two days and six days in a 5% CO<sub>2</sub> atmosphere and 100% humidity. 25

After incubation, the medium was removed and the cells were fixed in 0.1 ml of 10% trichloroacetic acid at 40°C. The plates were rinsed five times with deionized water, dried, stained for 30 minutes with 0.1 ml of 0.4% sulforhodamine B dye (Sigma) dissolved in 1% acetic acid, rinsed four times with 1% acetic acid to remove unbound dye, dried, and the stain was extracted for five minutes with 0.1 ml of 10 mM Tris base [tris(hydroxymethyl)aminomethane], pH 10.5. The absorbance (OD) of sulforhodamine B at 492 nm was measured using a computer-interfaced, 96-well microtiter plate reader. 30

A test sample is considered positive if it shows at least 50% growth inhibitory effect at one or more concentrations. The positive results are shown in the following Table 7.

Table 7

	<u>Compound</u>	<u>Tumor Type</u>	<u>Designation</u>
	PRO301	NSCL	NCI-H322M
	PRO301	Leukemia	MOLT-4; SR
	PRO301	NSCL	A549/ATCC; EKVX;
5	PRO301	NSCL	NCI-H23; NCI-460; NCI-H226
	PRO301	Colon	COLO 205; HCC-2998;
	PRO301	Colon	HCT-15; KM12; HT29;
	PRO301	Colon	HCT-116
	PRO301	CNS	SF-268; SF-295; SNB-19
10	PRO301	Melanoma	MALME-3M; SK-MEL-2;
	PRO301	Melanoma	SK-MEL-5; UACC-257
	PRO301	Melanoma	UACC-62
	PRO301	Ovarian	IGROV1; OVCAR-4
	PRO301	Ovarian	OVCAR-5
15	PRO301	Ovarian	OVCAR-8; SKOV-3
	PRO301	Renal	ACHN; CAKI-1; TK-10; UO-31
	PRO301	Prostate	PC-3; DU-145
	PRO301	Breast	NCI/ADR-RES; HS 578T
	PRO301	Breast	MDA-MB-435; MDA-N; T-47D
20	PRO301	Melanoma	M14
	PRO301	Leukemia	CCRF-CEM; HL-60(TB); K-562
	PRO301	Leukemia	RPMI-8226
	PRO301	Melanoma	LOX IMVI
	PRO301	Renal	786-0; SN12C
25	PRO301	Breast	MCF7; MDA-MB-231/ATCC
	PRO301	Breast	BT-549
	PRO301	NSCL	HOP-62
	PRO301	CNS	SF-539
30	PRO301	Ovarian	OVCAR-3

The results of these assays demonstrate that the positive testing PRO polypeptides are useful for inhibiting neoplastic growth in a number of different tumor cell types and may be used therapeutically therefor. Antibodies against these PRO polypeptides are useful for affinity purification of these useful polypeptides. Nucleic acids encoding these PRO polypeptides are useful for the recombinant preparation of these polypeptides.

#### EXAMPLE 68: Gene Amplification in Tumors

This example shows that certain PRO polypeptide-encoding genes are amplified in the genome of certain human lung, colon and/or breast cancers and/or cell lines. Amplification is associated with overexpression of the gene product, indicating that the polypeptides are useful targets for therapeutic intervention in certain cancers such as colon, lung, breast and other cancers and diagnostic determination of the presence of those cancers. Therapeutic agents may take the form of antagonists of the PRO polypeptide, for example, murine-human chimeric, humanized or human antibodies against a PRO polypeptide.

The starting material for the screen was genomic DNA isolated from a variety cancers. The DNA is quantitated precisely, *e.g.*, fluorometrically. As a negative control, DNA was isolated from the cells of ten normal healthy individuals which was pooled and used as assay controls for the gene copy in healthy

individuals (not shown). The 5' nuclease assay (for example, TaqMan™) and real-time quantitative PCR (for example, ABI Prizm 7700 Sequence Detection System™ (Perkin Elmer, Applied Biosystems Division, Foster City, CA)), were used to find genes potentially amplified in certain cancers. The results were used to determine whether the DNA encoding the PRO polypeptide is over-represented in any of the primary lung or colon cancers or cancer cell lines or breast cancer cell lines that were screened. The primary lung cancers were obtained from individuals with tumors of the type and stage as indicated in Table 8. An explanation of the abbreviations used for the designation of the primary tumors listed in Table 8 and the primary tumors and cell lines referred to throughout this example are given below.

The results of the TaqMan™ are reported in delta (Δ) Ct units. One unit corresponds to 1 PCR cycle or approximately a 2-fold amplification relative to normal, two units corresponds to 4-fold, 3 units to 8-fold amplification and so on. Quantitation was obtained using primers and a TaqMan™ fluorescent probe derived from the PRO polypeptide-encoding gene. Regions of the PRO polypeptide-encoding gene which are most likely to contain unique nucleic acid sequences and which are least likely to have spliced out introns are preferred for the primer and probe derivation, e.g., 3'-untranslated regions. The sequences for the primers and probes (forward, reverse and probe) used for the PRO polypeptide gene amplification analysis were as follows:

PRO533 (DNA49435-1219)

forward: 5'-GGGACGTGCTTCTACAAGAACAG-3' (SEQ ID NO:140)

reverse: 5'-CAGGCTTACAATGTTATGATCAGACA-3' (SEQ ID NO:141)

probe: 5'-TATTCAGAGTTTCCATTGGCAGTGCCAGTT-3' (SEQ ID NO:142)

PRO187 (DNA27864-1155)

forward: 5'-GGCCTTGCAGACAACCGT-3' (SEQ ID NO:143)

reverse: 5'-CAGACTGAGGGAGATCCGAGA-3' (SEQ ID NO:144)

probe: 5'-GCAGATTTTGAGGACAGCCACCTCCA-3' (SEQ ID NO:145)

forward2: 5'-CATCAAGCGCCTCTACCA-3' (SEQ ID NO:146)

reverse2: 5'-CACAAACTCGAACTGCTTCTG-3' (SEQ ID NO:147)

probe2: 5'-CAGCTGCCCTTCCCCAACCA-3' (SEQ ID NO:148)

PRO246 (DNA35639-1172)

forward: 5'-GGCAGAGACTTCCAGTCACTGA-3' (SEQ ID NO:149)

reverse: 5'-GCCAAGGGTGGTGTAGATAGG-3' (SEQ ID NO:150)

probe: 5'-CAGGCCCCCTTGATCTGTACCCCA-3' (SEQ ID NO:151)

The 5' nuclease assay reaction is a fluorescent PCR-based technique which makes use of the 5' exonuclease activity of Taq DNA polymerase enzyme to monitor amplification in real time. Two oligonucleotide primers (forward [f] and reverse [r]) are used to generate an amplicon typical of a PCR reaction. A third oligonucleotide, or probe (p), is designed to detect nucleotide sequence located between the two PCR primers. The probe is non-extendible by Taq DNA polymerase enzyme, and is labeled with a reporter fluorescent dye and a quencher fluorescent dye. Any laser-induced emission from the reporter dye is quenched by the quenching dye when the two dyes are located close together as they are on the probe.



During the amplification reaction, the Taq DNA polymerase enzyme cleaves the probe in a template-dependent manner. The resultant probe fragments disassociate in solution, and signal from the released reporter dye is free from the quenching effect of the second fluorophore. One molecule of reporter dye is liberated for each new molecule synthesized, and detection of the unquenched reporter dye provides the basis for quantitative interpretation of the data.

5           The 5' nuclease procedure is run on a real-time quantitative PCR device such as the ABI Prism 7700™ Sequence Detection. The system consists of a thermocycler, laser, charge-coupled device (CCD) camera and computer. The system amplifies samples in a 96-well format on a thermocycler. During amplification, laser-induced fluorescent signal is collected in real-time through fiber optics cables for all 96 wells, and detected at the CCD. The system includes software for running the instrument and for analyzing  
10           the data.

5' Nuclease assay data are initially expressed as Ct, or the threshold cycle. This is defined as the cycle at which the reporter signal accumulates above the background level of fluorescence. The  $\Delta C_t$  values are used as quantitative measurement of the relative number of starting copies of a particular target sequence in a nucleic acid sample when comparing cancer DNA results to normal human DNA results.

15           Table 8 describes the stage, T stage and N stage of various primary tumors which were used to screen the PRO polypeptide compounds of the invention.

Table 8  
Primary Lung and Colon Tumor Profiles

	Primary Tumor Stage	Stage	Other Stage	Dukes Stage	T Stage	N Stage
5	Human lung tumor AdenoCa (SRCC724) [LT1]	IIA			T1	N1
	Human lung tumor SqCCa (SRCC725) [LT1a]	IIB			T3	N0
	Human lung tumor AdenoCa (SRCC726) [LT2]	IB			T2	N0
	Human lung tumor AdenoCa (SRCC727) [LT3]	IIIA			T1	N2
	Human lung tumor AdenoCa (SRCC728) [LT4]	IB			T2	N0
10	Human lung tumor SqCCa (SRCC729) [LT6]	IB			T2	N0
	Human lung tumor Aden/SqCCa (SRCC730) [LT7]	IA			T1	N0
	Human lung tumor AdenoCa (SRCC731) [LT9]	IB			T2	N0
	Human lung tumor SqCCa (SRCC732) [LT10]	IIB			T2	N1
	Human lung tumor SqCCa (SRCC733) [LT11]	IIA			T1	N1
15	Human lung tumor AdenoCa (SRCC734) [LT12]	IV			T2	N0
	Human lung tumor AdenoSqCCa (SRCC735)[LT13]	IIB			T2	N0
	Human lung tumor SqCCa (SRCC736) [LT15]	IB			T2	N0
	Human lung tumor SqCCa (SRCC737) [LT16]	IB			T2	N0
	Human lung tumor SqCCa (SRCC738) [LT17]	IIB			T2	N1
20	Human lung tumor SqCCa (SRCC739) [LT18]	IB			T2	N0
	Human lung tumor SqCCa (SRCC740) [LT19]	IB			T2	N0
	Human lung tumor LCCa (SRCC741) [LT21]	IIB			T3	N1
	Human lung AdenoCa (SRCC811) [LT22]	IA			T1	N0
	Human colon AdenoCa (SRCC742) [CT2]	M1	D	pT4	N0	
25	Human colon AdenoCa (SRCC743) [CT3]			B	pT3	N0
	Human colon AdenoCa (SRCC744) [CT8]			B	T3	N0
	Human colon AdenoCa (SRCC745) [CT10]			A	pT2	N0
	Human colon AdenoCa (SRCC746) [CT12]		MO, R1	B	T3	N0
	Human colon AdenoCa (SRCC747) [CT14]		pMO, RO	B	pT3	pN0
30	Human colon AdenoCa (SRCC748) [CT15]		M1, R2	D	T4	N2
	Human colon AdenoCa (SRCC749) [CT16]		pMO	B	pT3	pN0
	Human colon AdenoCa (SRCC750) [CT17]			C1	pT3	pN1
	Human colon AdenoCa (SRCC751) [CT1]		MO, R1	B	pT3	N0
	Human colon AdenoCa (SRCC752) [CT4]			B	pT3	M0
35	Human colon AdenoCa (SRCC753) [CT5]		G2	C1	pT3	pN0
	Human colon AdenoCa (SRCC754) [CT6]		pMO, RO	B	pT3	pN0
	Human colon AdenoCa (SRCC755) [CT7]		G1	A	pT2	pN0
	Human colon AdenoCa (SRCC756) [CT9]		G3	D	pT4	pN2
	Human colon AdenoCa (SRCC757) [CT11]			B	T3	N0
40	Human colon AdenoCa (SRCC758) [CT18]		MO, RO	B	pT3	pN0

#### DNA Preparation:

DNA was prepared from cultured cell lines, primary tumors, normal human blood. The isolation was performed using purification kit, buffer set and protease and all from Quiagen, according to the manufacturer's instructions and the description below.

#### *Cell culture lysis:*

Cells were washed and trypsinized at a concentration of  $7.5 \times 10^8$  per tip and pelleted by centrifuging at 1000 rpm for 5 minutes at 4°C, followed by washing again with 1/2 volume of PBS recentrifugation. The pellets were washed a third time, the suspended cells collected and washed 2x with PBS. The cells were then suspended into 10 ml PBS. Buffer C1 was equilibrated at 4°C. Qiagen protease #19155 was diluted into 6.25 ml cold ddH<sub>2</sub>O to a final concentration of 20 mg/ml and equilibrated at 4°C. 10 ml of G2 Buffer was prepared

by diluting Qiagen RNase A stock (100 mg/ml) to a final concentration of 200  $\mu$ g/ml.

Buffer C1 (10 ml, 4°C) and ddH<sub>2</sub>O (40 ml, 4°C) were then added to the 10 ml of cell suspension, mixed by inverting and incubated on ice for 10 minutes. The cell nuclei were pelleted by centrifuging in a Beckman swinging bucket rotor at 2500 rpm at 4°C for 15 minutes. The supernatant was discarded and the nuclei were suspended with a vortex into 2 ml Buffer C1 (at 4°C) and 6 ml ddH<sub>2</sub>O, followed by a second 4°C centrifugation at 2500 rpm for 15 minutes. The nuclei were then resuspended into the residual buffer using 200  $\mu$ l per tip. G2 buffer (10 ml) was added to the suspended nuclei while gentle vortexing was applied. Upon completion of buffer addition, vigorous vortexing was applied for 30 seconds. Qiagen protease (200  $\mu$ l, prepared as indicated above) was added and incubated at 50°C for 60 minutes. The incubation and centrifugation was repeated until the lysates were clear (e.g., incubating additional 30-60 minutes, pelleting at 3000 x g for 10 min., 4°C).

*Solid human tumor sample preparation and lysis:*

Tumor samples were weighed and placed into 50 ml conical tubes and held on ice. Processing was limited to no more than 250 mg tissue per preparation (1 tip/preparation). The protease solution was freshly prepared by diluting into 6.25 ml cold ddH<sub>2</sub>O to a final concentration of 20 mg/ml and stored at 4°C. G2 buffer (20 ml) was prepared by diluting DNase A to a final concentration of 200 mg/ml (from 100 mg/ml stock). The tumor tissue was homogenated in 19 ml G2 buffer for 60 seconds using the large tip of the polytron in a laminar-flow TC hood in order to avoid inhalation of aerosols, and held at room temperature. Between samples, the polytron was cleaned by spinning at 2 x 30 seconds each in 2L ddH<sub>2</sub>O, followed by G2 buffer (50 ml). If tissue was still present on the generator tip, the apparatus was disassembled and cleaned.

Quiagen protease (prepared as indicated above, 1.0 ml) was added, followed by vortexing and incubation at 50°C for 3 hours. The incubation and centrifugation was repeated until the lysates were clear (e.g., incubating additional 30-60 minutes, pelleting at 3000 x g for 10 min., 4°C).

*Human blood preparation and lysis:*

Blood was drawn from healthy volunteers using standard infectious agent protocols and citrated into 10 ml samples per tip. Quiagen protease was freshly prepared by dilution into 6.25 ml cold ddH<sub>2</sub>O to a final concentration of 20 mg/ml and stored at 4°C. G2 buffer was prepared by diluting RNase A to a final concentration of 200  $\mu$ g/ml from 100 mg/ml stock. The blood (10 ml) was placed into a 50 ml conical tube and 10 ml C1 buffer and 30 ml ddH<sub>2</sub>O (both previously equilibrated to 4°C) were added, and the components mixed by inverting and held on ice for 10 minutes. The nuclei were pelleted with a Beckman swinging bucket rotor at 2500 rpm, 4°C for 15 minutes and the supernatant discarded. With a vortex, the nuclei were suspended into 2 ml C1 buffer (4°C) and 6 ml ddH<sub>2</sub>O (4°C). Vortexing was repeated until the pellet was white. The nuclei were then suspended into the residual buffer using a 200  $\mu$ l tip. G2 buffer (10 ml) were added to the suspended nuclei while gently vortexing, followed by vigorous vortexing for 30 seconds. Quiagen protease was added (200  $\mu$ l) and incubated at 50°C for 60 minutes. The incubation and centrifugation was repeated until the lysates were clear (e.g., incubating additional 30-60 minutes, pelleting at 3000 x g for 10 min., 4°C).

*Purification of cleared lysates:*

(1) Isolation of genomic DNA:

Genomic DNA was equilibrated (1 sample per maxi tip preparation) with 10 ml QBT buffer. QF elution buffer was equilibrated at 50°C. The samples were vortexed for 30 seconds, then loaded onto equilibrated tips and drained by gravity. The tips were washed with 2 x 15 ml QC buffer. The DNA was eluted into 30 ml silanized, autoclaved 30 ml Corex tubes with 15 ml QF buffer (50°C). Isopropanol (10.5 ml) was added to each sample, the tubes covered with parafin and mixed by repeated inversion until the DNA precipitated. Samples were pelleted by centrifugation in the SS-34 rotor at 15,000 rpm for 10 minutes at 4°C. The pellet location was marked, the supernatant discarded, and 10 ml 70% ethanol (4°C) was added. Samples were pelleted again by centrifugation on the SS-34 rotor at 10,000 rpm for 10 minutes at 4°C. The pellet location was marked and the supernatant discarded. The tubes were then placed on their side in a drying rack and dried 10 minutes at 37°C, taking care not to overdry the samples.

After drying, the pellets were dissolved into 1.0 ml TE (pH 8.5) and placed at 50°C for 1-2 hours. Samples were held overnight at 4°C as dissolution continued. The DNA solution was then transferred to 1.5 ml tubes with a 26 gauge needle on a tuberculin syringe. The transfer was repeated 5x in order to shear the DNA. Samples were then placed at 50°C for 1-2 hours.

(2) Quantitation of genomic DNA and preparation for gene amplification assay:

The DNA levels in each tube were quantified by standard  $A_{260}$ ,  $A_{280}$  spectrophotometry on a 1:20 dilution (5  $\mu$ l DNA + 95  $\mu$ l ddH<sub>2</sub>O) using the 0.1 ml quartz cuvetts in the Beckman DU640 spectrophotometer.  $A_{260}/A_{280}$  ratios were in the range of 1.8-1.9. Each DNA samples was then diluted further to approximately 200 ng/ml in TE (pH 8.5). If the original material was highly concentrated (about 700 ng/ $\mu$ l), the material was placed at 50°C for several hours until resuspended.

Fluorometric DNA quantitation was then performed on the diluted material (20-600 ng/ml) using the manufacturer's guidelines as modified below. This was accomplished by allowing a Hoeffer DyNA Quant 200 fluorometer to warm-up for about 15 minutes. The Hoechst dye working solution (#H33258, 10  $\mu$ l, prepared within 12 hours of use) was diluted into 100 ml 1 x TNE buffer. A 2 ml cuvette was filled with the fluorometer solution, placed into the machine, and the machine was zeroed. pGEM 3Zf(+) (2  $\mu$ l, lot #360851026) was added to 2 ml of fluorometer solution and calibrated at 200 units. An additional 2  $\mu$ l of pGEM 3Zf(+) DNA was then tested and the reading confirmed at 400 +/- 10 units. Each sample was then read at least in triplicate. When 3 samples were found to be within 10% of each other, their average was taken and this value was used as the quantification value.

The fluorometrically determined concentration was then used to dilute each sample to 10 ng/ $\mu$ l in ddH<sub>2</sub>O. This was done simultaneously on all template samples for a single TaqMan plate assay, and with enough material to run 500-1000 assays. The samples were tested in triplicate with Taqman™ primers and probe both B-actin and GAPDH on a single plate with normal human DNA and no-template controls. The diluted samples were used provided that the CT value of normal human DNA subtracted from test DNA was +/- 1 Ct. The diluted, lot-qualified genomic DNA was stored in 1.0 ml aliquots at -80°C. Aliquots which were subsequently to be used in the gene amplification assay were stored at 4°C. Each 1 ml aliquot is enough

for 8-9 plates or 64 tests.

*Gene amplification assay:*

The PRO polypeptide compounds of the invention were screened in the following primary tumors and the resulting  $\Delta C_t$  values greater than or equal to 1.0 are reported in Table 9 below.

Table 9  
 $\Delta$ Ct values in lung and colon primary tumors and cell line models

	Primary Tumors or Cell Lines	PRO187	PRO533	PRO246
5	LT7		1.04	
	LT13	2.74 2.98 2.44		1.63 1.68
10	LT3			1.06
	LT12	2.70 2.90		2.47 1.74
15		2.27		
	LT30	1.67		
	LT21			1.50
20	LT-1a		1.02	
	LT10			1.07
25	LT11		1.09	3.43 1.41
	LT15	3.75 3.92 3.49		2.11 1.56
30	LT16	2.10	1.66	
	LT17		1.32	2.68 1.69
35	LT19	4.05 3.99	1.67	1.91 1.68 1.16
40	CT2	3.56		
	CT8	1.01		
45	CT10	1.81		
	CT14	1.82		
	CT1	1.24 1.34		
50	CT5	2.96 2.99		1.33 2.39

Table 9 (cont')  
ΔCt values in lung and colon primary tumors and cell line models

Primary Tumors or Cell Lines	PRO187	PRO533	PRO246
CT6	1.10		
CT7	1.40		
CT9	1.39		1.09
CT11	2.22		1.48
	2.26		1.12

Because amplification of the various DNAs described above occurs in various cancerous tumors and tumor cell lines derived from various human tissues, these molecules likely play a significant role in tumor formation and/or growth. As a result, amplification and/or enhanced expression of these molecules can serve as a diagnostic for detecting the presence of tumor in an individual and antagonists (*e.g.*, antibodies) directed against the proteins encoded by the above described DNA molecules would be expected to have utility in cancer therapy.

**EXAMPLE 69: Gene Expression in Bovine Pericytes (Assay 105)**

This assay is designed to identify gene expression patterns in pericytes induced by the hits in assay 93 described above. Bovine pericytes are plated on 60mm culture dishes in growth media for 1 week. On day 1, various PRO polypeptides are diluted (1 %) and incubated with the pericytes for 1, 4 and 24 hr. timepoints. The cells are harvested and the RNA isolated using TRI-Reagent following the included instructions. The RNA is then quantified by reading the 260/280 OD using a spectrophotometer. The gene expression analysis is done by TaqMan reactions using Perkin Elmer reagents and specially designed bovine probes and primers. Expression of the following genes is analyzed: GAPDH, beta-integrin, connective tissue growth factor (CTGF), ICAM-1, monocyte chemoattractant protein-1 (MCP-1), osteopontin, transforming growth factor-beta (TGF-beta), TGF-beta receptor, tissue inhibitor of metalloproteinase (TIMP), tissue factor (TF), VEGF- $\alpha$ , thrombospondin, VEGF- $\beta$ , angiopoietin-2, and collagenase. Replicates are then averaged and the SD determined. The gene expression levels are then normalized to GAPDH. These are then normalized to the expression levels obtained with a protein (PIN32) which does not significantly induce gene expression in bovine pericytes when compared to untreated controls. Any PRO polypeptide that gives a gene expression level 2-fold or higher over the PIN32 control is considered a positive hit.

The following PRO polypeptides tested positive in this assay: PRO217.

**EXAMPLE 70: Cytokine Release Assay (Assay 120)**

This assay is designed to determine whether PRO polypeptides of the present invention are capable

of inducing the release of cytokines from peripheral blood mononuclear cells (PBMCs). PRO polypeptides capable of inducing the release of cytokines from PBMCs are useful from the treatment of conditions which would benefit from enhanced cytokine release and will be readily evident to those of ordinary skill in the art. Specifically,  $1 \times 10^6$  cells/ml of peripheral blood mononuclear cells (PBMC) are cultured with 1% of a PRO polypeptide for 3 days in complete RPMI media. The supernatant is then harvested and tested for increased concentrations of various cytokines by ELISA as compared to a human IgG treated control. A positive in the assay is a 10-fold or greater increase in cytokine concentration in the PRO polypeptide treated sample as compared to the human IgG treated control.

The following polypeptides tested positive in this assay: PRO9940.

**EXAMPLE 71: Identification of PRO Polypeptides That Activate Pericytes (Assay 125)**

This assay shows that certain polypeptides of the invention act to activate proliferation of pericyte cells and, therefore, are useful not only as diagnostic markers for particular types of pericyte-associated tumors but also for giving rise to antagonists which would be expected to be useful for the therapeutic treatment of pericyte-associated tumors. Activation of pericyte proliferation also correlates with the induction of angiogenesis and, as such, PRO polypeptides capable of inducing pericyte proliferation would be expected to be useful for the treatment of conditions where induced angiogenesis would be beneficial including, for example, wound healing, and the like. Specifically, on day 1, pericytes are received from VEC Technologies, and all but 5 ml media is removed from the flask. On day 2, the pericytes are trypsinized, washed, spun and plated on 96 well plates. On day 7, the media is removed and the pericytes are treated with  $100 \mu\text{l}$  of either the specific PRO polypeptide or control treatments (positive control = DME+5% +/- PDGF @  $500\text{ng}/\mu\text{l}$ ; negative control = PIN32, a polypeptide determined to have no significant effect on pericyte proliferation). C-fos and GAPDH gene expression levels are then determined and the replicates are averaged and the SD is determined. The c-fos values are normalized to GAPDH and the results are expressed as fold increase over PIN2. Anything providing at least a 2-fold or higher response as compared to the negative control is considered positive for the assay.

The following polypeptides tested positive in this assay: PRO217.

**EXAMPLE 72: Identification of Receptor/Ligand Interactions**

In this assay, various PRO polypeptides are tested for ability to bind to a panel of potential receptor or ligand molecules for the purpose of identifying receptor/ligand interactions. The identification of a ligand for a known receptor, a receptor for a known ligand or a novel receptor/ligand pair is useful for a variety of indications including, for example, targeting bioactive molecules (linked to the ligand or receptor) to a cell known to express the receptor or ligand, use of the receptor or ligand as a reagent to detect the presence of the ligand or receptor in a composition suspected of containing the same, wherein the composition may comprise cells suspected of expressing the ligand or receptor, modulating the growth of or another biological or immunological activity of a cell known to express or respond to the receptor or ligand, modulating the immune response of cells or toward cells that express the receptor or ligand, allowing the preparation of agonists,



antagonists and/or antibodies directed against the receptor or ligand which will modulate the growth of or a biological or immunological activity of a cell expressing the receptor or ligand, and various other indications which will be readily apparent to the ordinarily skilled artisan.

The assay is performed as follows. A PRO polypeptide of the present invention suspected of being a ligand for a receptor is expressed as a fusion protein containing the Fc domain of human IgG (an immunoadhesin). Receptor-ligand binding is detected by allowing interaction of the immunoadhesin polypeptide with cells (e.g. Cos cells) expressing candidate PRO polypeptide receptors and visualization of bound immunoadhesin with fluorescent reagents directed toward the Fc fusion domain and examination by microscope. Cells expressing candidate receptors are produced by transient transfection, in parallel, of defined subsets of a library of cDNA expression vectors encoding PRO polypeptides that may function as receptor molecules. Cells are then incubated for 1 hour in the presence of the PRO polypeptide immunoadhesin being tested for possible receptor binding. The cells are then washed and fixed with paraformaldehyde. The cells are then incubated with fluorescent conjugated antibody directed against the Fc portion of the PRO polypeptide immunoadhesin (e.g. FITC conjugated goat anti-human-Fc antibody). The cells are then washed again and examined by microscope. A positive interaction is judged by the presence of fluorescent labeling of cells transfected with cDNA encoding a particular PRO polypeptide receptor or pool of receptors and an absence of similar fluorescent labeling of similarly prepared cells that have been transfected with other cDNA or pools of cDNA. If a defined pool of cDNA expression vectors is judged to be positive for interaction with a PRO polypeptide immunoadhesin, the individual cDNA species that comprise the pool are tested individually (the pool is "broken down") to determine the specific cDNA that encodes a receptor able to interact with the PRO polypeptide immunoadhesin.

In another embodiment of this assay, an epitope-tagged potential ligand PRO polypeptide (e.g. 8 histidine "His" tag) is allowed to interact with a panel of potential receptor PRO polypeptide molecules that have been expressed as fusions with the Fc domain of human IgG (immunoadhesins). Following a 1 hour co-incubation with the epitope tagged PRO polypeptide, the candidate receptors are each immunoprecipitated with protein A beads and the beads are washed. Potential ligand interaction is determined by western blot analysis of the immunoprecipitated complexes with antibody directed towards the epitope tag. An interaction is judged to occur if a band of the anticipated molecular weight of the epitope tagged protein is observed in the western blot analysis with a candidate receptor, but is not observed to occur with the other members of the panel of potential receptors.

Using these assays, the following receptor/ligand interactions have been herein identified:

- (1) PRO533 binds to the fibroblast growth factor receptor-4 (FGFR-4; see Partanen et al., EMBO J. 10(6):1347-1354 (1991)).
- (2) PRO301 binds to itself and, therefore, functions as an adhesion molecule.
- (3) PRO187 binds to the fibroblast growth factor receptor-3 (FGFR-3; see Keegan et al., Proc. Natl. Acad. Sci. USA 88:1095-1099 (1991)) with high affinity and with lower affinity to FGFR-1, 2 and 4 (see Isacchi et al., Nuc. Acids Res. 18(7):1906 (1990), Dionne et al., EMBO J. 9(9):2685-2692 (1990) and Partanen et al., EMBO J. 10(6):1347-1354 (1991), respectively).

- (4) PRO337 binds to PRO6004.
- (5) PRO1411 binds to PRO4356.
- (6) PRO10096 binds to PRO2630.
- (7) PRO246 binds to itself and, therefore, functions as an adhesion molecule.
- (8) PRO6307 binds to PRO265.
- 5 (9) PRO6003 binds to PRO941.

#### Deposit of Material

The following materials have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, USA (ATCC):

10

Table 10

	<u>Material</u>	<u>ATCC Dep. No.</u>	<u>Deposit Date</u>
	DNA22779-1130	209280	September 18, 1997
	DNA26846-1397	203406	October 27, 1998
15	DNA32279-1131	209259	September 16, 1997
	DNA32288-1132	209261	September 16, 1997
	DNA33094-1131	209256	September 16, 1997
	DNA33785-1143	209417	October 28, 1997
	DNA35663-1129	209201	June 18, 1997
20	DNA46777-1253	209619	February 5, 1998
	DNA60783-1611	203130	August 18, 1998
	DNA62306-1570	203254	September 9, 1998
	DNA62880-1513	203097	August 4, 1998
	DNA64896-1539	203238	September 9, 1998
25	DNA71290-1630	203275	September 22, 1998
	DNA96031-2664	PTA-237	June 15, 1999
	DNA108722-2743	PTA-552	August 17, 1999
	DNA35674-1142	209416	October 28, 1997
	DNA41234	209618	February 5, 1998
30	DNA77503-1686	203362	October 20, 1998
	DNA49435-1219	209480	November 21, 1997
	DNA40628-1216	209432	November 7, 1997
	DNA27864-1155	209375	October 16, 1997
	DNA43316-1237	209487	November 21, 1997
35	DNA59212-1627	203245	September 9, 1998
	DNA86576-2595	203868	March 23, 1999
	DNA35639-1172	209396	October 17, 1997
	DNA36350-1158	209378	October 16, 1997
	DNA53906-1368	209747	April 7, 1998
40	DNA125185-2806	PTA-1031	December 7, 1999
	DNA83568-2692	PTA-386	July 20, 1999

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These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposits will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted

availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC § 122 and the Commissioner's rules pursuant thereto (including 37 CFR § 1.14 with particular reference to 886 OG 638).

5           The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

10           The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the construct deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any  
15           aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.